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Studies on Seed Production and Vernalization of Cruciferous Crops in the Tropics

By
Tzay-Fa SHEEN

Fengshan Tropical Horticultural Experiment Station, TARI
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by

TZAY-FA SHEEN*

Abstract

In the tropics, the temperature is not low enough to induce or promote flowering of most cruciferous crops, except in the highland. Highland seed production faces difficulties however due to frequent rain in the season. This study was carried out to evaluate the methods of vernalization for seed production of crucifers in the tropics and substropics.

Using the phasic development theory, cruciferous crops were grown and vernalized in the highlands and then the mother plants were transplanted to the warmer and drier lowlands for photo-phase development. This technique made possible the successful seed production of cabbage and kohlrabi in Taiwan. Results also showed that different altitudes provided the necessary cold requirement for phasic development in seed production of cruciferous crops.

The investigation also revealed that long period of seed production under insufficient cold environment was the principal cause of the degeneration of cruciferous varieties in the tropics. Premature bolting is a major degraduation problem for most varieties. Therefore, slow bolting is a desirable trait for varietal development. The use of vernalization method to induce flowering enables the selection for non-bolting or slow bolting lines. To complete the phasic cycle of the slow bolting lines. Stock-seed plants of Peking Late kohlrabi and Ta-Mei-Fua Late radish were exposed to low temperature of 8 ± 1℃ for six weeks and 9 ± 1℃ for three weeks, respectively. Thus, the seed production of these varieties became possible.

The metabolic changes in carbohydrate and nitrogen compounds during the vernalization of mature plants were almost identical for the green-plant vernalization type (kohlrabi) and the seed-vernalization type (radish). The sugar content of the shoot apex increased during the vernalization period and reached the plateau just before flower primordium initiation, and then decreased during flower bud development stage. The number of tunica layers also increased when the sugar content reached a maximum and thereafter decreased, matching the timing of the transition from a vegetative meristem to a floral meristem.

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However, the vernalization of seed showed difference in the change of sugar content between the green-plant vernalization type and the seed-vernalization type. The seed-vernalization type showed an increase in total sugar and reducing sugar at the beginning of the low temperature treatment. The green-plant vernalization type, by comparison, showed no change in sugar content during vernalization, but later showed the same changes in sugar content as seed-vernalization type when the plant had grown to a certain stage at which it was responsive to low temperature.

High day temperatures annul the effect of low night temperature, thus, causing devernalization. However, with complete vernalization, the plants maintained stable high sugar contents which could not be nullified by subsequent high temperature or alternation of high day/low night temperatures. Hence, devernalization did not occur after the vernalization was finished. For the seed production in the tropics, therefore care should be taken in growing plants in certain locations to insure that devernalization is prevented. It is also very important to finish the vernalization completely in order to have smooth seed production.

Thus, the use of highland and lowland areas to provide the essential requirements of the first- and second-phasic development of varieties not only resolved the insufficiently low temperature of the lowland tropics but also avoided flower and seed production during rainy season in the highland areas and devernalization problems in the lowland. Furthermore selection of the required type of plant and head (root or swollen stem) characters as stock-seed plant by means of vernalization can be incorporated easily into the process of breeding and seed production thereby preventing premature bolting and degeneration of varieties. The above techniques enabled the seed production of cruciferous crops and provision of good varieties to growers in the tropics.

General Introduction

Cabbage *(Brassica oleracea* L. var. *Capitata* L.), Kohlrabi *(Brassica olaracea* L. var. *gongylodes* L.) and radish *(Raphanus sativus* L.) are generally cultivated for their head leaves, swollen stem, and root respectively. These salable products, however, are not essential in the seed germination to seed production cycle. Large scale commercial seed production methods are normally different from those used in stock seed production for breeding. The seed grower does not require the formation of head, swollen stem, or root for flowering, whereas for the latter, head, swollen stem, or root maturity is necessary for selection purpose.

In the tropics, the temperatures required for flower induction are not low enough or do not last long enough for seed production of slow bolting varieties. It then becomes necessary to import seeds of these varieties from temperate countries. Thus for varieties which have the potential to produce seed in tropical or subtropical areas, it is necessary to develop seed production and flower promoting technology.

Normally, seed production of cabbage and radish, the two major cruciferous crops in Taiwan, is impossible or unstable for those varieties which require a long period of low temperature treatment. Nearly 100% of the cabbage seeds in Taiwan are imported. Seed yield of late radish varieties is low, and problems of premature bolting arise from these seedlots due to a long term, insufficient cold stimulus, Thus, the technique of promoting flowering in slow bolting plants is the most problematic aspect of the seed production cycle.

Seed production is sometimes possible at high altitudes in tropical and subtropical regions, but there are still difficulties. Lee and Shen (1961) conducted seed production tests with Kimmen Early kohlrabi at different altitudes and found that at high altitudes, flowering and bolting occurred in the early summer. The seeds were still ripening, however, when the rainy season began from June to July which made seed production impossible. Lee (1966) evaluated seed production at high altitudes in Taiwan for several vegetables including cabbage, Chinese cabbage, and carrot. Although the low temperature conditions were comparable to those found in temperate regions and sufficient for flower induetion, the rainy season again hampered seed production. Sheen (1970, 1972, 1975) conducted breeding and seed production work with cabbage at the Taiwan Seed Improvement Station of the Provincial Department of Agriculture and Forestry, from 1969 to 1975, and pointed out that their genetic materials were limited to only a few varieties of early bolting types such as cabbage var. Yehsen.

Since Lysenko established the "phasic development theory" from his flower induction studies with wheat, a number of studies have examined the relationship between low temperature and bolting with cruciferous plants such as seedling sensitivity, varietal differences in response to low temperatures, and ecological and genecological differentiation (Eguchi and Koide 1944; Fukushima snd Kamimoto 1950; Boswell 1929; Watanabe 1954; Shinohara 1959; Yamasaki 1962). Following Lysenko's study, a number of reports indicated that fowering of most cruciferous crops is accelerated by high temperatures and long day conditions after vernalization was complete (Sugiyama 1944; Shinohara 1948; 1959; Miller 1929). These studies suggested many vernalization techniques that might be practically applied in tropical and subtropical areas.

There are many studies on the metabolic changes during vernalization regarding sugar content, RNA, and auxins (Kato 1965; Ogata and Sugiyama 1968; Suge et al, 1968; Suzuki 1972). A study on the metablic changes in some cruciferous crops during vernalization was conducted by the author from 1966 to 1967 under the guidance of Professor Koya Yamasaki and Dr. Yoshio Suzuki at Tokyo University of Education. Almost all of these studies, however, have been concerned with the metabolic changes occuring between vernalization and flower initation, and not so much from flower initiation to flowering. Shinohara (1959) according to the phasic development of cruciferous plants, divided the sensitive stage into at least two types: Seed-vernalization type is able to respond to low temperature at the seed germination stage; the n = 10 and n = 19 groups of *Brassica* spp. and radish belong to this type. Green-plant vernalization type is able to respond to low temperature at green seedling stage, or after having grown to a certain degree, and n = 9 group of *Brassica* spp. belongs to this type. Physiological conditions of flower formation vary rather widely with differences among varieties and differences among strain of the same variety. Considerable range of variation was revealed in the bolting habit among strains or stocks in each variety. Seeds of Ta-Mei-Fua radish, for instance, are vernalized imperfectly as a population and if sown in the summer, the early bolting types will bolt in fall or even in summer of the same year, while the late bolting type will bolt in spring of the next year. The progenies of seeds selected from the early bolting plants bolt earlier than those derived from slow bolting plants. It is expected that by selecting non-bolting plants in the growing season as the stock-seed plants better progenies can be obtained. The experiment used Peking Late Kohlrabi as a green-plant vernalization type and

Ta-Mei-Fua Late radish as a seed-vernalization type to evaluate methods of promoting stock-seed plants to flower, and to study the morphological changes in the shoot apex and their relationship to carbohydrate and nitrogen compounds during vernalization and flowering.

The low temperature in the lowland of the tropics and subtropics is not low enough for flower induction. Moreover, the possibility of devernalization cause by high temperature is another factor limiting seed production. Interest in devernalization has led to studies not only of effects of high temperature following vernalization, but also of alternation of high temperature with low temperature during the period of vernalization (Gregory and Pruvis 1938, Suzuki 1981). This experiment was conducted to confirm the effect in the tropics of vernalization being erased by high temperature or by higher day temperature, and to examine the relationship of temperature changes to metabolic changes of sugar contents during devernalization.

The author discussed the seed production problems of cruciferous crops in the tropics along the following four areas: (1) insufficient winter chilling to promote flower development; (2) overlap of seed production with rainy season impairing pollination and seed development; (3) devernalization due to high temperature; (4) and degeneration of varieties into early bolting types due to the perpetuation of premature bolting plants through several years of seed production under unstable climatic conditions. The studies reported here were carried out to investigate the following:

(1) use of highland and lowland areas to complete the phasic development requirement of crucifer varieties in order that flowering and seed production will be smooth;
(2) improvement of technique of stock-seed plant vernalization in the procedure of seed production and breeding and its related physiological changes in carbohydrate and nitrogen compounds;
(3) analysis of devernalization and its related physiological problems.

The above studies were designed to develop the correct principle of seed production and breeding in tropics.

I. Seed production and vernalization of cabbage in the subtropics

A. Introduction

Seed production of common cabbage in the tropics and subtropics is difficult because of insufficient winter chilling to promote flower development. Most common cabbage varieties in Taiwan remain in the vegetative stage throughout the winter season. The local var. Yehsen cabbage (Early bolting type) does not require as low a temperature as other cabbage varieties to induce flowering easily, and therefore can produce seed in the lowland subtropics. Insufficient low temperatures, however, induce only incomplete flowering, and thus seed yields differ from year to year and are much lower than those obtained in temperate regions. At high elevations, the winter chilling is sufficient to attain complete flowering, but the cabbage grows much slower and therefore flowers into the rainy season. Heavy rains prevent flower pollination, and the cabbage then rots (Lee 1966).

Most seed-vernalization and green-plant vernalization cruciferae require long days and warm temperatures for bolting and flowering after vernalization (Sugiyama 1942; Eguchi and Koide 1944; Shinohara 1959). Seed ripening also requires dry conditions. The southern and southwestern coastal areas of Taiwan have warm temperatures and a low relative humidity in the winter season. These conditions can be favorable for bolting and ripening seeds of common cabbage after vernalization. The purposes of this experiment were to determine the optimal planting time for Yehsen cabbage seed production at Taiwan Seed Service (500m above sea level), and for completely vernalized plants transplanted in the southern and southwestern coastal areas. Timing for seed production of those varieties requiring a deep degree of the requirement to low temperature was also evaluated with crops planted first in highland areas and then transplanted to lowland areas. The results may provide some guidelines for common cabbage seed production in the tropics and subtropics.

B. Materials and methods

(1) Early bolting type: var. Yehsen cabbage

a. Yehsen cabbage sown at different times

Seeds were sown in seedling flats at Taiwan Seed Service (500m above sea level) on September 24, October 13 and

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November 1, 1975. One-month-old seedlings were randomized complete block design with 3 replications and transplated to the field. Plant were observed for days-to-first-flower, and the number of leaves and stem diameter at flowering. If the plants formed their heads through winter, their heads were intersected to let them bolt. Seed yield of each treatment, and 1,000-seed weight, seed number per milliliter, seed germination rate were investigated by bulk from each treatment for replicate test.

b. Transplanting Yehsen cabbage to southern and southwestern coastal areas after vernalization.

Seed were sown in seedling flats on September 28, and then transplanted on October 24, 1975 at Taiwan Seed Service. (TSS). After heading, the heads were harvested on January 21, 1976 and the plants were dug out with root, then transferred to An-nan in Tainan city, Yen-Shui of Tainan county, Hu-wei of Yun-lin county, and the experimental farm of Taiwan Seed Service (Taichung county). The technique of axillary bud bolting was tried to evaluate the potential for seed production in these areas. Day-to-harvesting and seed quality were bulked from each production area for replicate test.

(2) Slow bolting type: var. Chu-Chiou cabbage

a. Seed production methods for K-Y cross Chu-Chiou cabbage after vernalization

Seeds were sown at the Wu-lin Farm (1700m above sea level) and at the lowland Fengshan Tropical Horticultural Experiment Station (FTHES) on June 15 and September 15, 1976 respectively. Plants at the Wu-lin Farm were vernalized for a certain period and then transplanted to FTHES on November 18, 1976. Both treatments were observed for flowering and seed production efficiency.

b. Transplant timing for K-Y cross Chu-Chiou cabbage

Cabbage seed were sown at Wu-lin Farm (1700m above sea level) on June 20, 1977. Headed plants were harvested in the middle of October and then left in the field for natural vernalization. The stems were then transplanted to the FTHES experimental farm on November 1 and December 8, 1977, and January 12, 1978 for flowering and seed production. To compare seed-to-seed and headed plant-to-seed methods of seed production, seeds were sown on

September 22, 1977 at the FTHES experimental farm. One-month-old seedlings were transplanted to the Wu-lin Farm on November 3 for vernalization, and those plants which did not form heads were returned to FTHES on December 20 for evaluation of flowering and seed production.

C. Results and Discussion

a. Seed production of Yehsen cabbage sown at different times

Plants sown on September 24, 1975 headed completely, whereas plants sown on later dates headed only partially. Leaf number and stem size at flowering are shown in Table 1–1. The average leaf number and stem size of the first planting were greater than those of the later planting dates. Plants of the first and second plantings flowered on February 26 and 28, 1976 respectively, and the third planting flowered on March 20, 1976. Plants of the second sowing had the highest seed yield bearing large-sized seeds, and plants of the third sowing had the lowest seed yield (Table 1–2). Both 1,000-seed weight and seed germination rates followed the same pattern as seed yield.

Table 1–1. *Flowering behavior of Yehsen cabbage under different sowing dates*

Sowing date	Date of flowering*	Days from sowing to flowering	No. of leaves at flowering	Size of stem at flowering (cm)
9/24	2/26	182	51.75	2.98
10/13	2/28	151	36.00	2.63
11/1	3/20	140	32.00	2.19

*Date on which half of the plants had flowered

Table 1–2. *Sowing time and quantity and quality of Yehsen cabbage seed*

Sowing date	Seed yield (g/plant)	1,000-seed weight (g)	No. of seed/ml	Germination rate (%)
9/24	21.40^{b*}	4.05^{b}	162.3^{b}	87.8^{a}
10/13	29.03^{a}	4.50^{a}	159.0^{c}	88.5^{a}
11/1	14.03^{c}	3.20^{c}	210.5^{a}	83.2^{b}

* Mean in each vertical column followed by same letter are not significantly different at 5% level using Duncans' multiple range test

Commercial common cabbage seed production in the temperate regions usually does not progress through head fromation, whereas in the tropics and subtropics, plants remain vegetative because of insufficiently low temperatures. Even plants planted at TSS in low temperatures averaging 14.8°C in late winter or early spring have to head to seed normally. Therefore, seed-to-seed methods cannot be employed as in the temperate regions. However, cabbage heads were cut and used for seed production. Axillary buds were used to prevent pest infestation. Plants of the first sowing were larger and had a greater soft-rot infestation rate at the time of cutting than the other two sowings. Average seed production was thus lower. Plants of the third sowing did not flower until March 20, and as a result of gradually increasing temperatures and the approaching rainy season, seed production was the lowest among the three treatments.

b. Yehsen cabbage seed production at different southern and southwestern coastal locations after vernalization

Plants transplanted at An-nan, Yen-shui and Hu-wei began flowering in February and were comparable with those plants remaining at TSS. During the flowering period, the average temperature and relative humidity at An-nan were 19.5°C, 72%, Yen-shui 18.6°C, 78%, Hu-wei 17.9°C, 72%, Taiwan Seed Service 16.9°C, 81.1% (Table 1–3, Fig. 1–1, 1–2).

Seeds of those plants transplanted in southern and southwestern coastal areas were harvested at the end of April. Plants transplanted at Hu-wei, Yen-shui and An-nan of coastal areas had higher seed yields, 1,000-seed weights and large-sized seeds compared with those plants remaining at TSS (Table 1–4).

Table 1–4. *Quantity and quality of Yehsen cabbage seed produced in different areas*

Production area	Date of seed harvest	1,000-seed weight (g)	No. of seed/ml	Germination rate (%)
An-nan	4/26	4.29^{ab*}	160.5^{b}	95.2^{a}
Yen-shui	5/1	4.23^{ab}	155.6^{c}	95.0^{a}
Hu-wei	4/30	4.43^{a}	158.8^{bc}	$94\ 2^{a}$
Taiwan seed service	4/25	2.85^{c}	234.0^{a}	89.3^{b}

* Mean in each vertical column follow by same letter are not significantly different at 5% level using Duncans' multiple range test

Table 1–3. *Climate data from different seed production areas*

Areas and locations	Weather variable*	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Taiwan													
Seed	M.T. (°C)	14.2	15.3	18.5	21.9	25.3	25.4	26.5	25.2	25.2	22.7	19.6	16.6
Service	T.P. (mm)	23.8	81.4	98.4	109	222	430	299	295	237	42.8	24.3	20.9
(Taichung county)	T.S. (hr)	92.5	93.4	102	100	103	96.0	94.2	86.4	93.6	100	138	116
Hu-wei (Yun-lin county)	M.T. (°C)	15.5	16.3	19.4	22.4	24.9	26.8	28.0	27.2	26.4	23.5	19.9	17.6
	T.P. (mm)	35.9	23.3	50.0	44.7	198	214	190	311	45.2	11.4	17.3	12.7
	T.S. (hr)	158	132	133	139	133	174	216	135	167	184	138	177
Yen-shui	M.T. (°C)	16.1	16.8	20.3	23.8	25.3	27.8	29.3	28.9	28.8	25.1	21.6	18.1
(Tainan county)	T.P. (mm)	34.6	12.2	34.3	43.6	153	242	266	331	32.3	10.1	8.3	5.7
An-nan (Tainan city)	M.T. (°C)	16.9	17.9	21.0	23.7	25.4	27.5	27.9	27.3	26.9	24.7	20.9	18.9
	T.P. (mm)	20.7	10.9	22.4	52.6	177	442	340	348	74.2	17.5	21.9	3.9
	T.S. (hr)	155	148	164	154	137	166	177	150	159	183	151	154
Fengshan (Kaohsiung county)	M.T. (°C)	18.3	19.3	21.6	24.8	27.4	28.3	28.9	28.6	28.1	26.4	23.5	20.3
	T.P. (mm)	21.9	16.7	35.1	44.6	154	394	308	337	190	60.8	9.1	9.8
	T.S. (hr)	170	155	165	193	178	379	200	159	155	172	142	171
Lishan	M.T. (°C)	7.55	9.13	12.3	14.9	17	18.8	19.6	18.7	17.2	14.3	11.6	9.3
	T.P. (mm)	79.9	86.2	138	108	240	181	96.5	397	102	124	54.7	56.9

*M.T. : mean temperature, T.P. : total precipitation, T.S. : total sunshine

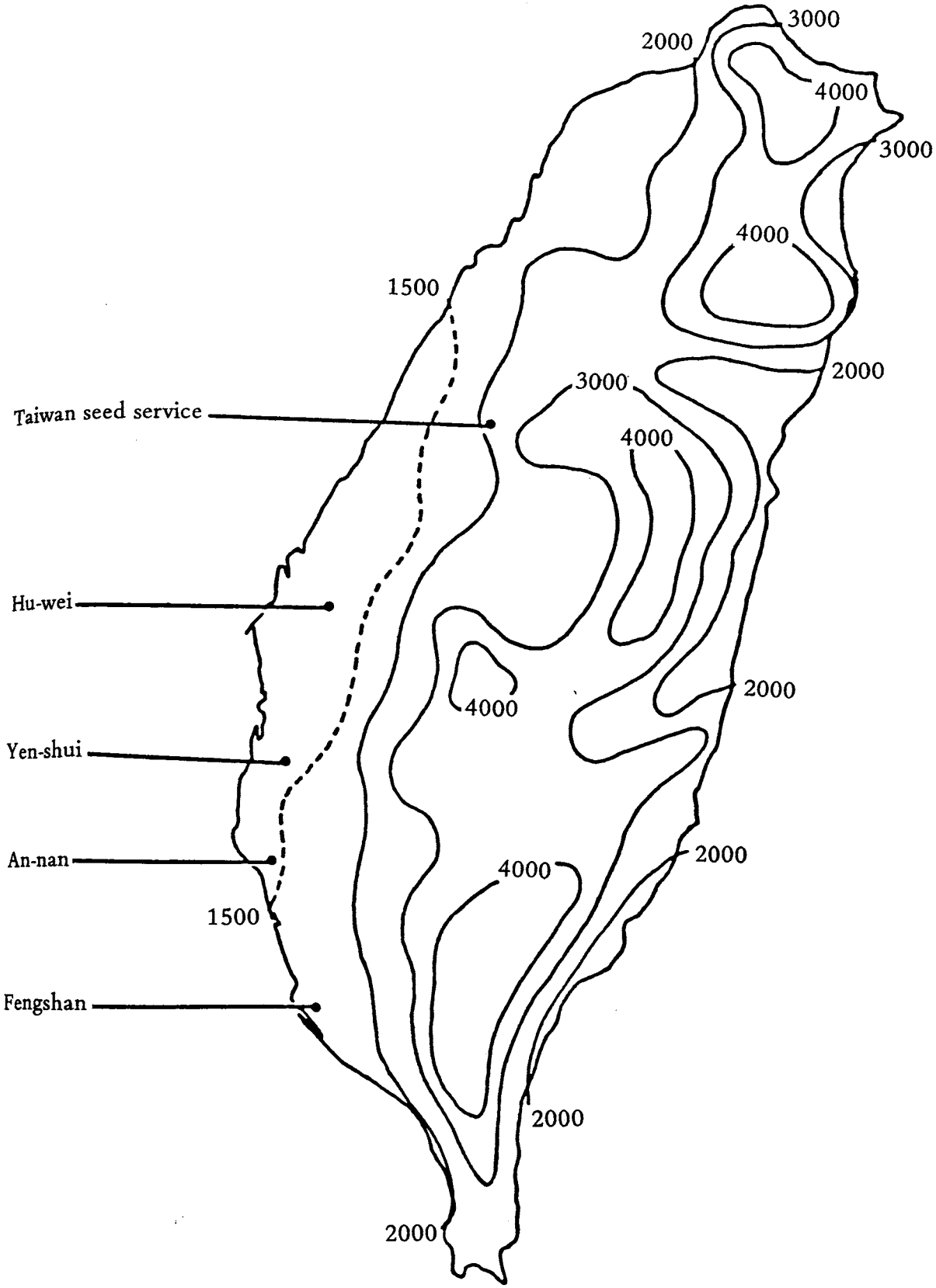

Fig. 1–1. Annual total precipitation (mm) in different areas of Taiwan.

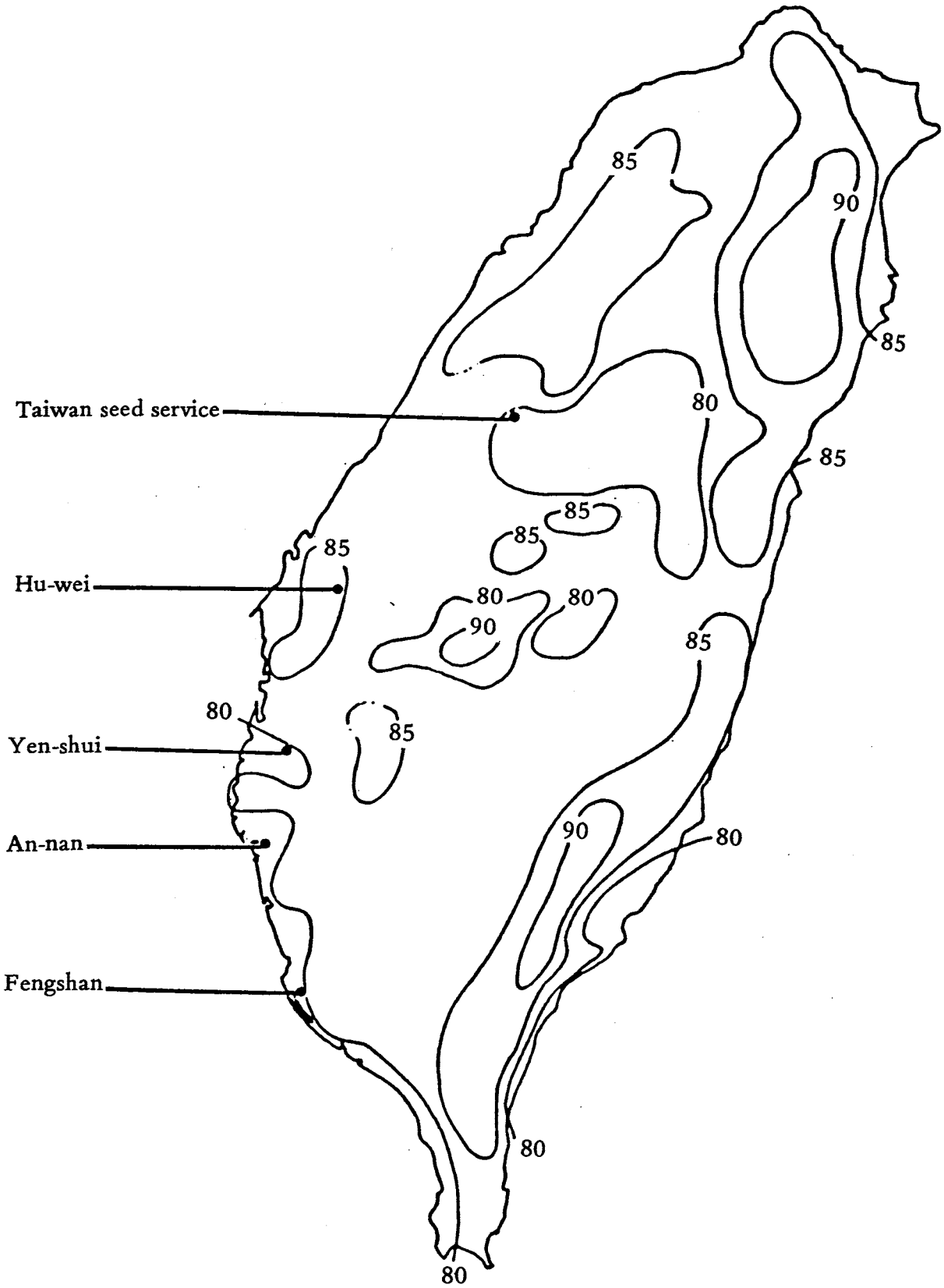

Fig. 1–2. Annual mean relative humidity (%) in different areas of Taiwan.

The 1,000-seed weight and number of seeds/ml of the seed harvested in the coastal areas were compared with those of imported seed. The results indicate that relatively dry weather has great influence on seed quality.

c. Seed production methods for K-Y cross Chu-Chiou cabbage after vernalization

K-Y cross Chu-Chiou cabbage does not flower when planted in southern Taiwan. In this experiment, plants sown in the middle of September at FTHES and the heads cut of on December 24 did not flower until the following March. On the other hand, plants grown in the highlands (heads harvested in October, and transplanted at FTHES in November) began flowering on December 18 and completed flowering on January 28 (Fig. 1–3). Seeds were harvested on March 15 (Table 1–5). Seed qualities of 1,000-seed weight and seed number per milliliter did not differ from those of imported seed.

Fig. 1–3. Comparison of flowering behavior of Chu-Chiou cabbage.
Left – planted in lowland.
Right – transplanted to lawland after vernalization in highland.

Table 1–5. *Seed production method and quantity and quality of K-Y cross Chu-Chiou Cabbage seed.*

Seed production method	Date* of flowering	Date of seed harvest	Seed** yield (g/plant)	1,000-seed weight (g)	No. of seed/ml
Transplanted to lowland after vernalization in highland	12/18	3/15	18.8	5.45	112.5
Planted in lowland	–	–	–	–	–

*Date on which half of the plant had flowered
**Average of 20 plants

After plants were transplanted to the lowlands, the warm and dry conditions of the southern parts of Taiwan were favorable for accelerating flowering and seed ripening after vernalization. Average temperatures in December, January and February were 20.3°C, 18.3°C and 19.3°C respectively, mean relative humidity was less than 80% and daylengths were long. Seeds were harvested before the rainy season. Seed harvesting for Yehsen cabbage planted at TSS is done normally at the end of April. By vernalizing headed plants in the highlands and then transplanting to the lowlands for seed production, seeds can be harvested up to one and a half months earlier than is possible when using traditional production methods.

d. Transplant timing for K-Y cross Chu-Chiou cabbage

Different transplanting dates significantly influenced flowering time. Plants vernalized perfectly demonstrated earlier or quicker flowering. The first transplanting of headed vernalized plants (November 1) flowered in 30 days. Plants of the second (December 8) and third (January 12) transplantings flowered in 12 and 8 days respectively (Table 1–6). It appears that perfect vernalization accelerated bolting and flowering time. Imperfectly vernalized plants from the first transplanting showed imperfect bolting and flowering. 1,000-seed weight and seed yield per plant from the first transplanting were the lowest among the four treatments. Plants from the second transplanting

Table 1–6. *Date of lowland transplanting and the quantity and quality of cabbage seed after high altitude vernalization*

Date of transplanting from hihgland to lowland	Date* of flowering	Date of seed harvest	Seed yield (g/plant)	1,000-seed weight (g)	No. of seed/ml	Remarks
11/1	11/30	3/3	7.50^{c**}	3.71^{d}	169.5^{a}	headed plant
12/8	12/20	3/15	17.00^{a}	5.56^{a}	106.4^{d}	to seed
1/12	1/20	3/27	8.56^{b}	4.65^{b}	129.7^{c}	
12/20	1/20	4/25	7.50^{c}	4.21^{c}	147.1^{b}	seed to seed

*Date on which half of the plants had flowered

**Mean in each vertical column follow by same letter are not significantly different at 5% level using Duncans' multiple range test

flowered perfectly, bearing large-sized seeds and producing high seed yields. Although plants from the third transplanting flowered perfectly, their longer stalks sustained more injuries during transport, resulting in small seed size and low seed yields. The non-heading, vernalized plants also had low 1,000-seed weight, low seed yield per plant and small-sized seed. The results, however, indicate that complate vernalization in the highlands may lead to normal flowering and seed production when plants are transplanted to the lowlands. Early to mid-December appeared to be the optimal time for transplanting.

Bowswell (1929) and Miller (1929) found that cabbage flowered when it over-wintered at the seedling stage in which the stem diameter was greater than 6mm. He also found that flowering was accelerated by high temperature conditions after the low temperature treatment. Watanabe (1954) pointed out that there were varietal differences in the sensitivity of seedlings to low temperature treatments. Sugiyama (1942), Eguchi and Koide (1944) and Shinohara (1948) evaluated Chinese cabbage and radish under different sowing times and concluded that the later the sowing time which still allowed for complete vernalization, the fewer the number of days to bolting. Their experiments showed that bolting and flowering after low tmeperature treatments were accelerated by long days and high temperatures.

Based on Lysenko-Whyte's phasic development theory,

shinohara (1955) illustrated the phasic development of several cruciferous crops. Cabbage is a green-plant vernalization type. In the lengths of the basic vegetative phase, the first phase (thermo-phase) and the second phase (photo-phase) were shown to differ with different cabbage varieties. In this experiment, different cabbage varieties were vernalized at different altitudes in order to complete perfectly the development of the first phase. After satisfactory exposure to low temperatures for vernalization, the plants were transplanted to the warmer and longer day conditions of the lowlands to accelerate the second phase development. From December to February, mean temperatures range from 18°C to 20°C and relative humidity is at its lowest (less than 80%) in southern Taiwan. These are good conditions for bolting and seed production. Too high a temperature, however, might result in the devernalization of the plant. Suzuki et al (1980) showed that alternating vernalized radish in an environment with high day temperature would prolong the period to bolting or prevented bolting altogether. However, if the plants complete the first phase of development (thermo-phase), the second phase (photo-phase) development can proceed smoothly under mild weather conditions. The method of transferring plants to favorable environmental conditions to adjust the development of the first and second phases, with its resulting smooth vernalization and flowering, appears ideal for cabbage seed production in the tropics and subtropics.

Perfect vernalization not only accelerated bolting and flowering, but also promoted complete seed setting (Shinohara 1950; Watanabe 1954). The problems of cabbage seed production in the tropics and subtropics were insufficiently low temperatures in the first phase and rain in the second phase. Thus, considering the varied conditions required for vernalization, flowering and seed ripening, the date when plants are transplanted from the highlands to the lowlands is crucial to the success of seed production. The experimental results present several different modes of cabbage seed production in the subtropics (Fig. 1–4). Modes I and II are produced in the lowland. Insufficiently low temperature was a problem for both headed plant-to-seed and seed-to-seed methods and resulted in unstable seed yields. Furthermore, lowland seed production limited the choice of variety to early bolting types such as var.

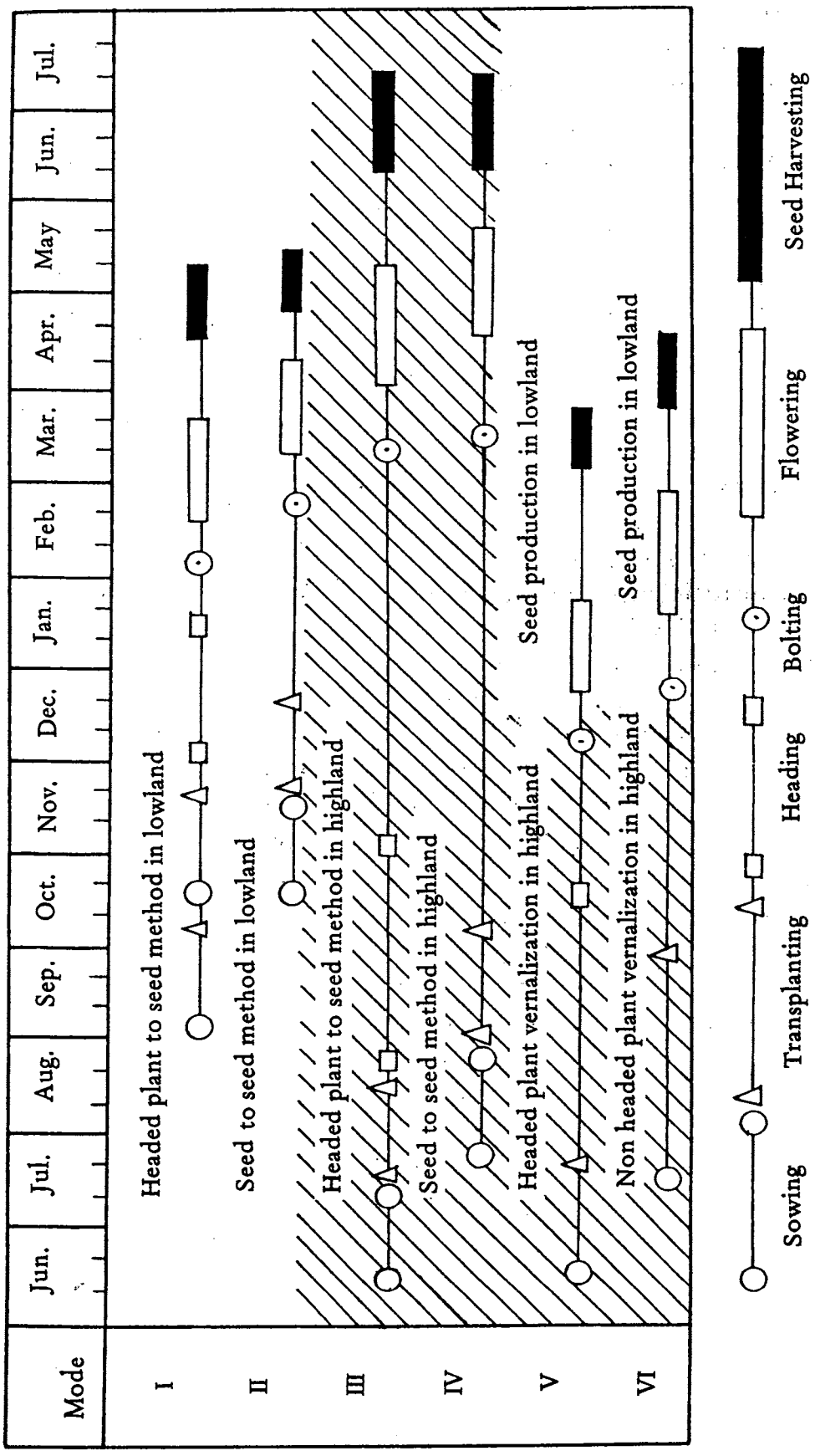

Fig. 1–4. Modes of cabbage seed production in the subtropics.

Yehsen. Both headed plant-to-seed and seed-to-seed plants in modes III and IV flowered during the rainy season. The low temperature conditions were sufficient for the first phase to develop completely, but flowering during the rainy season increased problems with pollination, soft-rot and sclerotinia rot resulting in failed seed production (Lee 1966). The first phase of Modes V and VI developed under low temperatures and the second phase was completed under warmer and drier conditions. Although the results from mode VI were not as good compared with those of mode V, further study on the exact seedling stage, duration of highland vernalization and transplanting time for the seed-to-seed method is required.

D. Summary

Low temperatures are essential for cabbage flower induction. Most cabbage varieties cannot bloom and produce seeds in the tropics because of insufficiently low temperatures. Some early bolting types may flower completely in the highlands but fail to produce seed during the subsequent rainy season. Accoring to the phasic development of cruciferous crops, plants were instead planted in the highlands for first phase (thermo-phase) development, and then transplanted to the lowlands for second phase (photo-phase) development.

The materials used were tropical Yehsen cabbage which requires only shallow low temperatures, and the northern K-Y cross Chu-Chiou cabbage which requires a deeper degree of low temperatures. The results show that flowering and seed ripening were accelerated by warmer and drier (or longer day) conditions after complete vernalization. Seed production is thus possible by using different altitudes in the tropics corresponding to the phasic requirement of cabbage.

II. Vernalization of slow bolting stock-seed plants in the tropics

In the subtropics or tropics, temperature are not low enough to induce flowering of most cruciferous crops. Varieties distributed in the subtropics or tropics belong to the early bolting type. Their degree of the cold requirement gradually changes from early types towards the extremely early or even chose lacking in it. Since environmental conditions differ from year to year, seed production is significatly affected by the climate of that year. Seed yield would be lower in a warm year. Premature bolting then become a serious problem and a variety may lose its value owing to gene elimination by discarding plants that did not flower.

Normally, seed productions were managed in the following three steps: 1) Foundation stock seed production or Elite stock seed. 2) Registered seed production or original seed. 3) Certified seed production or commercial seed.

The third step of commcial seed production could follow the phasic development of cruciferous crops, plants were vernalized in the cool highland and then transplanted to the warmer and drier lowland for seed production. But, the first and the second steps of stock seed production always included the selection process and multiplication of the foundation stock seed. In order to avoid the inbreeding depression occur in most cruciferous crops. Maternal-line selection method was aplied widely in stock seed production. Problems arise on the plants which have been selected for good horticultural characteristics and slow bolting as stock plants. How to induce these stock plants to flower becomes and important step in breeding and the process of seed production. Peking Late kohlrabi of green-plant vernalization type and Ta-Mei-Fua radish of seed-vernalization type were used in this experiment to study flower inducing methods and flowering behaviors of different types of cruciferous crops. The low temperature requirement of the above two varieties belong to slow bolting type. For inducing stock-seed plants of slow bolting varieties to flower in stock seed production and studying their morphological changes of the shoot apex and relationship of carbohydrate and nitrogen compounds during vernalization, the following three experiments were carrier out and discussed.

*1. Vernalization and flower development in green-plant vernalization type of Kohlrabi**

A. Introduction

Kohlrabi is a popular vegetable in Taiwan. The most commonly grown variety is "Peking Late Maturing". This variety, however, will not bolt and flower under the natural conditions of the Taiwan lowlands because of insufficiently low temperatures. The seed of this variety must therefore be imported. Kohlrabi is a "green-plant vernalization type", and the low temperatures of the temperate zones are usually sufficient to induce bolting and flowering. Since certain highland areas of Taiwan have temperate regions comparable to certain seasons in the temperate zones, the present project evaluated highland vernalization of kohlrabi (Peking Late Maturing) for initiation of flowering and facilitating seed production of this vegetable in Taiwan.

The bolting and flowering physiology of kohlrabi was studied in several experiments which subjected kohlrabi plants at different stages of plant growth to different degrees and durations of low temperatures. After identifying the optimal degree and duration of low temperature treatment, as well as the stage of plant growth for optimal seedling sensitivity, highland areas in Taiwan can be indentified for kohlrabi vernalization for seed production purposes.

After highland vernalization, the plants were transplanted in the lowlands to hasten seed production and thereby avoid the rainy season in May and June. Selected kohlrabi plants were vernalized for different durations, examined microscopically for floral differentiation and analyzed for biochemical changes in the swollen stem after vernalization.

B. Materials and Methods

(1) Effect of vernalization at different seedling stages

Kohlrabi seeds were sown on November 29, December 9 and December 18, 1977. Seedlings of three different ages (Table 2–1) were then simultaneously dug out on March 9, 1978 and placed in a humid growing room at 8°C and 12 hr day lengths. Seedlings were transplanted to the field after 18, 28 or 38 days of vernalization, and observed for flowering responses (Figure 2–1). A non-vernalized treatment was used as a control.

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* *Presented at Eucarpia "Crucifeae 1979" Conference, Wageningen, the Netherlands, and published at Journal of Agricultural Research of China 28(2), 1979.*

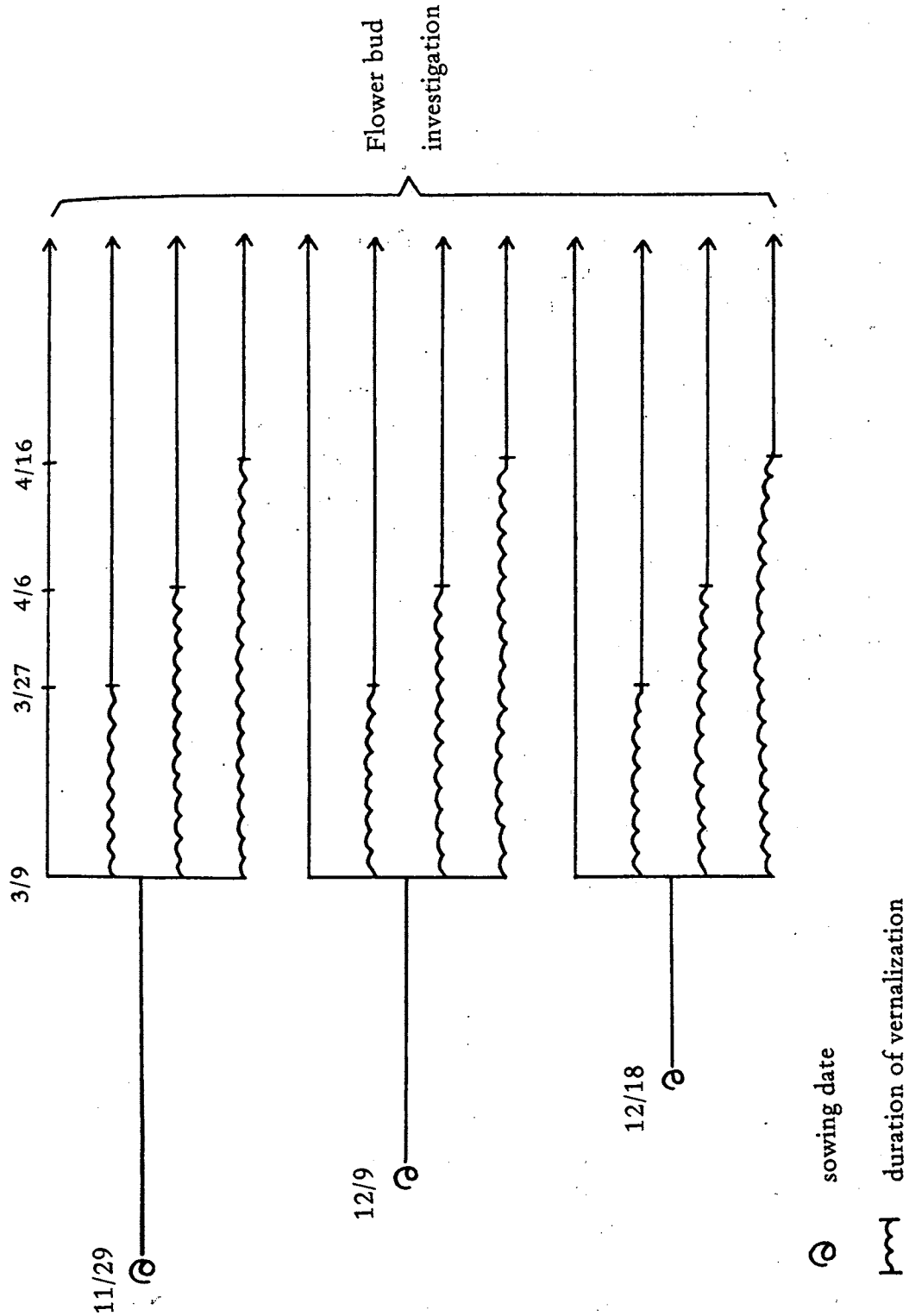


Fig. 2–1. Experimental plan on vernalization at different seedling stages.

(2) Effect of vernalization on flower initiation and metaboli changes of mature plants

Seeds were sown on September 1, 1977 and seedlings wer transplanted to the field on October 1, 1977. Plants were du out on December 3, after their stems had swollen. The leave were removed, and the plants were then placed in a humi growing room at 8°C and total darkness. Twenty plants wer removed from the room and transplanted to the field at weekl intervals beginning December 10 and continuing for seve weeks. Plants grown in the field were sampled and observed fo flowering responses (Figure 2–2).

Plant samples for carbohydrate and nitrogen determination were also taken each time. Plants were removed from the roon and transplanted to the field. The last plants were removec from the cold room on January 21. Random samples of singl swollen stems were taken from each treatment on February 1 and 8 to observe flower bud differentiation, and to determin nitrogen and carbohydrate contents. Total nitrogen was deter mined by the Kjeldahl method, soluble sugar by the Anthron method and reducing sugar by the Somogyi method.

(3) Highland vernalization

Seeds were sown on the lowland on September 9, 1977 and seedings were transported to highland Fu-shou-shan (2200m) for vernalization. Plants were returned to the lowland area on January 28 and February 18 and observed for flowering responses. More than 100 plants were observed in each verna lization treatment.

C. Results and Discussion

(1) Effect of vernalization at different seedling stages

The leaf number and stem diameter of the three different seedling stages at vernalization are shown in Table 2–1. For the seedlings planted on November 29, 66.6%, 100% and 100% flowering responses were observed for the 18, 28 and 38 day vernalization treatments respectively. No flowering was observed with the non-vernalized seedlings. For the seedlings planted on December 9, 83%, 100% and 100% flowering responses were observed for the 18, 28, and 38 day vernalization treatments respectively. No flowering was observed with the non-vernalized seedlings. For the seedlings planted on December 18, there was no flowering response regardless of vernalization duration (Table 2–2). These results indicate that the Peking Late

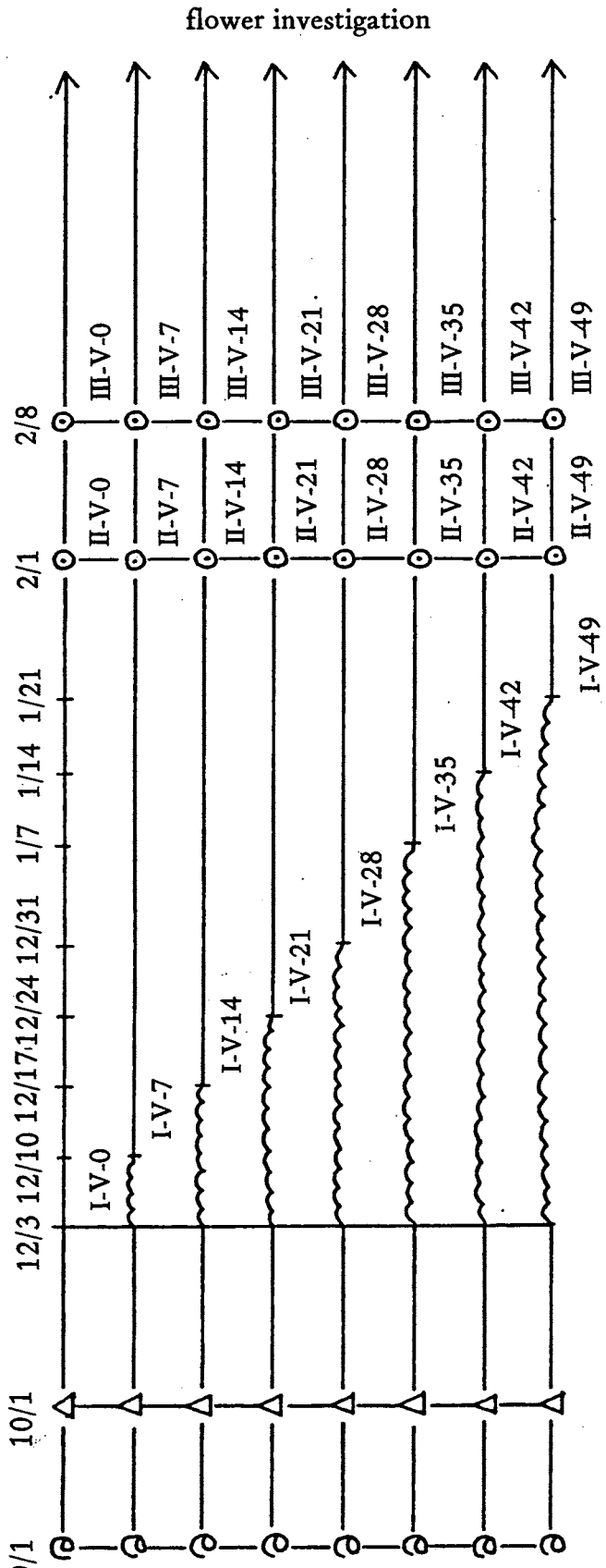


Fig. 2—2. Experimental plan for vernalization of mature plants

Table 2–1. *The status of different aged seedlings of "Peking Late Maturing" kohlrabi at the beginning of low temperature treatments (March 9)*[a]

Seedling group	Date of sowing	Days after sowing	Number of expanded leaves	Size of stem diameter (mm)
I	11/29	100	15.0	8.6
II	12/9	90	12.5	5.5
III	12/18	81	8.5	2.0

a. See figure 1 for reference

Table 2–2. *The bolting behavior of "Peking Late Maturing" kohlrabi as influenced by various durations of low temperature treatments (8°C) at different seedling stages*[a]

Seedling group	Duration of treatment (days)	Number of plants		Percent of plants flowered
		Treated	Bolted	
I	0	6	0	0
	18	6	4	67
	28	6	6	100
	38	6	6	100
II	0	6	0	0
	18	6	5	83
	28	6	6	100
	38	6	6	100
III	0	6	0	0
	18	6	0	0
	28	6	0	0
	38	6	0	0

a. See figure 1 for description of treatments

Maturing variety of kohlrabi needs to have at least 13 expanded leaves and a 6mm stem diameter in order to be sensitive to a one-month vernalization period at 8°C.

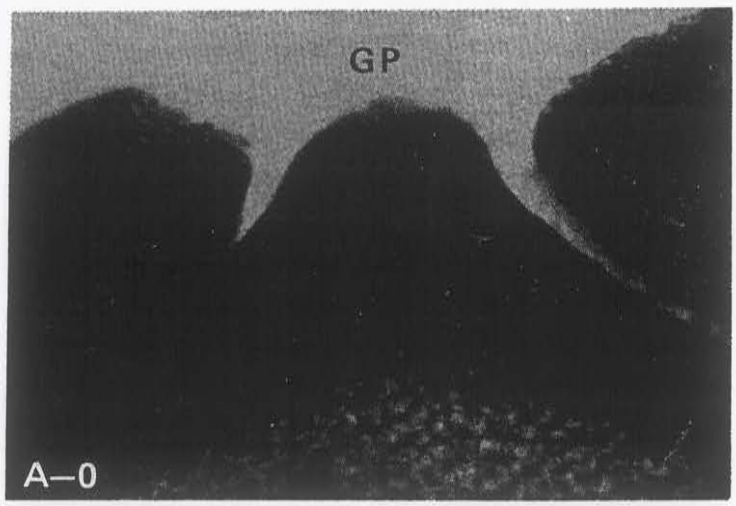
(2) Effect of vernalization on flower initiation and metabolic changes of mature plants

Observations through a microscope showed that the growing point of non-vernalized plants sampled on February 1 were

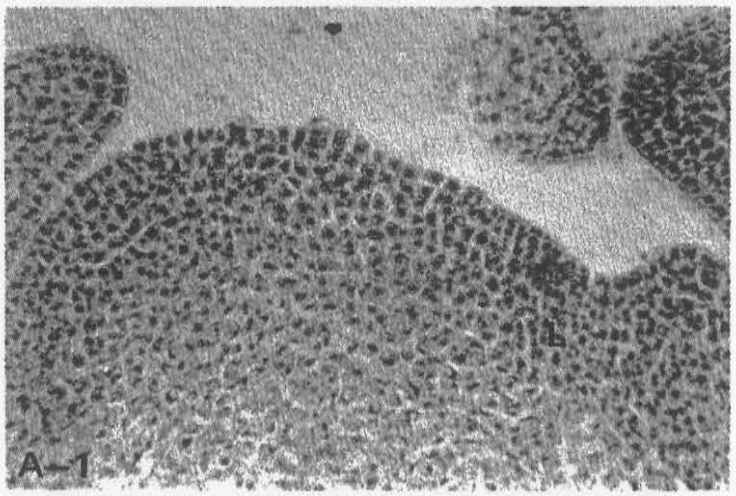
relatively flat and small, indicating that they were still in the vegetative stage (Figure 2–3: A–0). The growing points of plants which had been vernalized for 7 or 14 days were visibly swollen (Figure 2–3: A–1 and A–2) and revealed leaf primodium. Leaf primodium of those plants which had been vernalized for 21 days had already grown taller at the time of sampling (Figure 2–3: A–3), whereas leaf primodium of the plants vernalized for 28 days continued to grow (Figure 2–3: A–4). Flower buds were visible on those plants which had been vernalized for 35 to 49 days.

The growing points of non-vernalized plants sampled on February 8 had a flat apex (Figure 2–3: B–0), whereas growing points of those plants which had been vernalized for 7 or 14 days had a globular shaped apex (Figure 2–3: B–1 and B–2). Leaf primodium of plants vernalized for 21 days began to form on a bulge on the growing point at the time of sampling (Figure 2–3: B–3). Flower primordium still not initiation were clearly visible on plants vernalized for 28 days (Figure 2–3: B–4), whereas sepal and flower buds were completely differentiated on those plants vernalized for 35 to 42 days. Plants vernalized for 49 days began bolting at the time of sampling. All plants began to flower on February 15, with the exception of those without or with only short periods of vernalization. On March 15, flowering rates were 16.3%, 61.1%, 88.9%, and 94.6% for the 3, 5, 6, and 7 week vernalization treatments, respectively (Figure 2–4). The higher flowering rate of the plants vernalized for three weeks compared with plants vernalized for four weeks might be due to the fact that Peking Late Maturing kohlrabi is a fixed variety which is not uniform in genetic background. In General, however, plants with a longer duration of vernalization flowered more quickly with complete bolting and flowering, whereas plants with shorter vernalization periods either did not flower or flowered incompletely (Fig. 2–5).

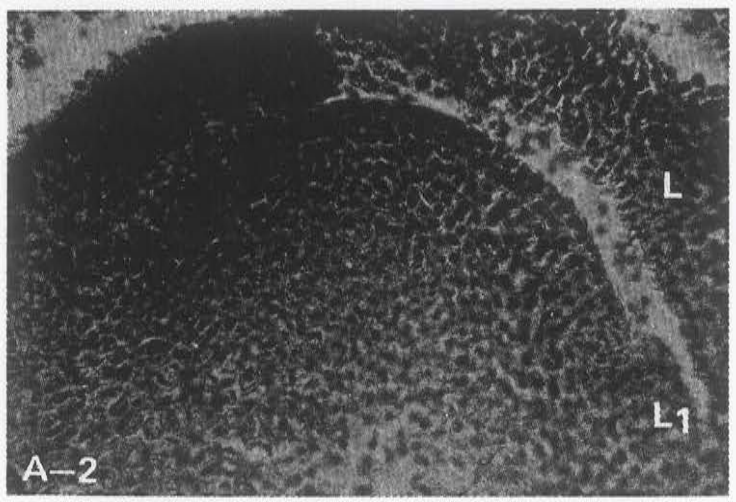
The changes in carbohydrates and nitrogen in the swollen stem after vernalization are shown in Table 2–4. There was no significant change in total nitrogen (I-V-0—I-V-49) during vernalization. Soluble nitrogen contents also did not change during the first three weeks of vernalization (I-V-0—I-V-21), but started to increase during the fourth week of vernalization (I-V-28—I-V-29). Total sugar, non-reducing sugar and reducing



A—0. Non-treat (Stage II-V-0) – Growing point (G P) raised. Vegetative adult.

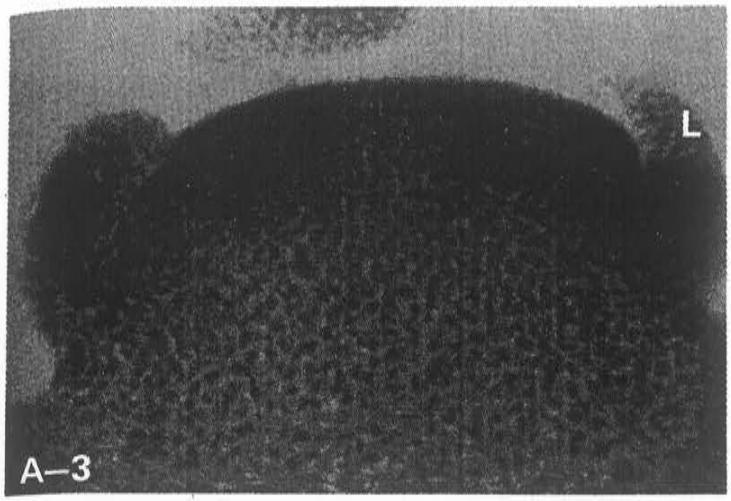


A—1. One-week treatment (Stage II-V-7) – Growing point globular and heavily stained cells where the leaf primodium was initated.

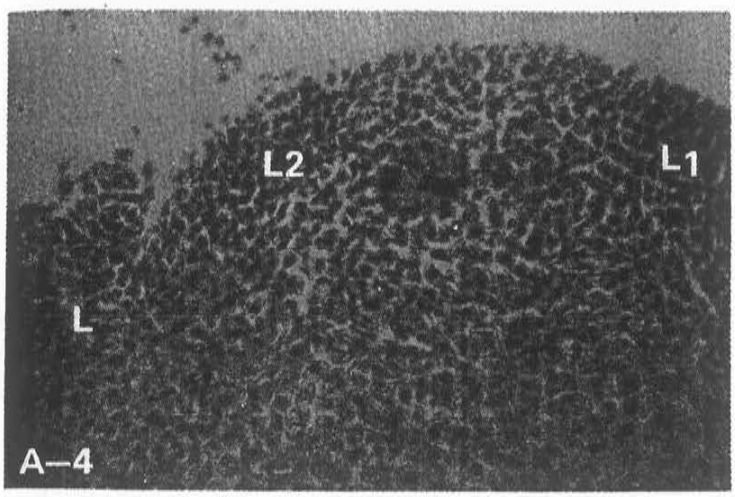


A—2. Two-weeks treatment (Stage II-V-14) – First leaf was formed (L_1).

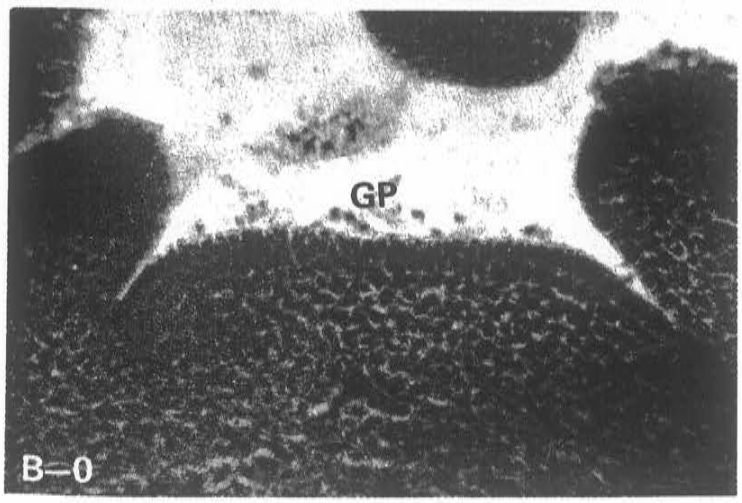
Fig. 2—3. Floral development the swollen stems of kohlrabi treated with different durations of low temperature (8°C). See Fig. 2 for reference of experimental plan. A. Investigated on Feb. 1, 1977. B. Investigated on Feb. 8, 1977.



A–3. Three weeks treatment (Stage II-V-21) – The apex gradually grew higher and thicker.

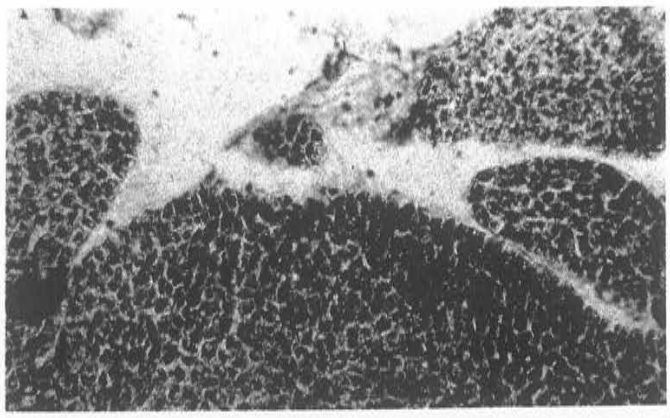


A–4. Four-weeks treatment (Stage II-V-28) – The width and height of apex were more developed. The heavily stained cells showed where the 1st and 2nd leaf primodia were initiated from terminal respectively.

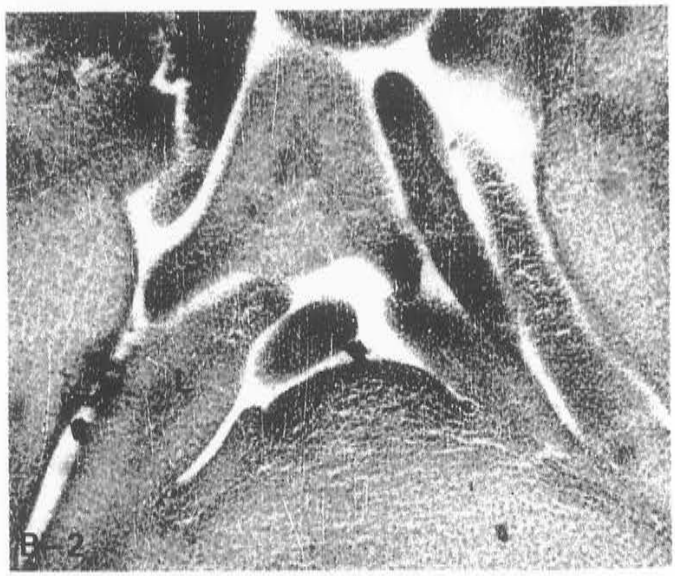


B–0. Non-treated (Stage III-V-0)—Flat apex.

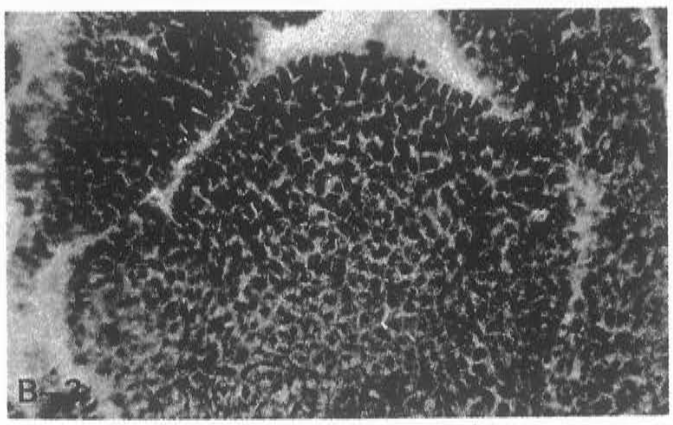
Fig. 2–3. Floral development the swollen stems of kohlrabi treated with different durations of low temperature (8°C). See Fig. 2 for reference of experimental plan. A. Investigated on Feb. 1, 1977. B. Investigated on Feb. 8, 1977.



B–1. One week treatment (Stage III-V-7) – growing point globular, no further development.

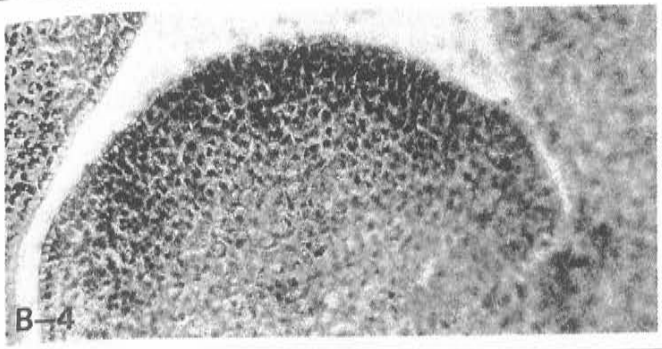


B–2. Two-weeks treatment (Stage III-V-14) – As same as B–1.

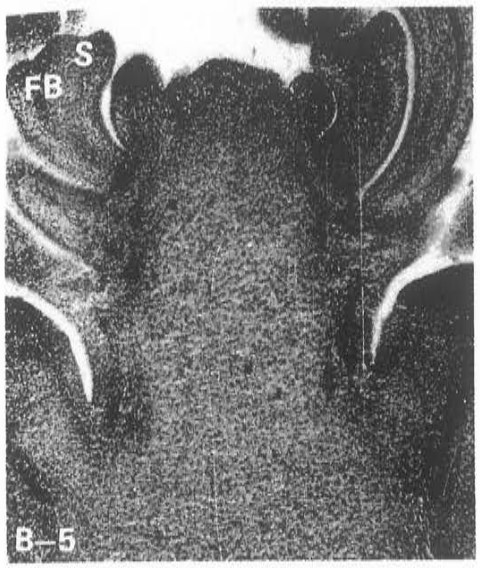


B–3. Three weeks treatment (II-V-21) – Leaf primodium has risen in axils of the leaf, but is still in the same stage as A–3.

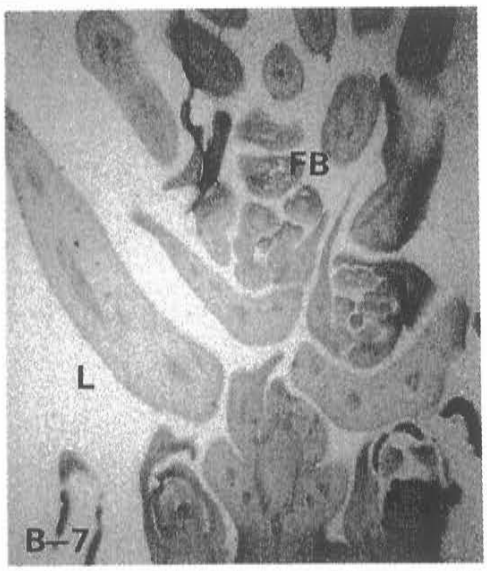
Fig. 2–3. Floral development the swollen stems of kohlrabi treated with different durations of low temperature (8°C). See Fig. 2 for reference of experimental plan. A. Investigated on Feb. 1, 1977. B. Investigated on Feb. 8, 1977.



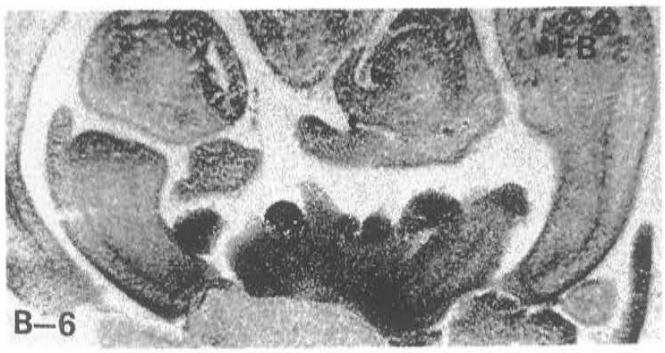
B–4. Four weeks treatment (III-V-28) – The cells at subapical region did not develop to form flower bud.



B–5. Five weeks treatment (III-V-35) – Flower bud (FB) and sepal (S) were formed.



B–7. Seven weeks treatment – Flower buds (FB) were developed completely and bolting.



B–6. Six weeks treatment – flower bud (FB) and sepal (S) were more developed.

Fig. 2–3. Floral development the swollen stems of kohlrabi treated with different durations of low temperature (8°C). See Fig. 2 for reference of experimental plan. A. Investigated on Feb. 1, 1977. B. Investigated on Feb. 8, 1977.

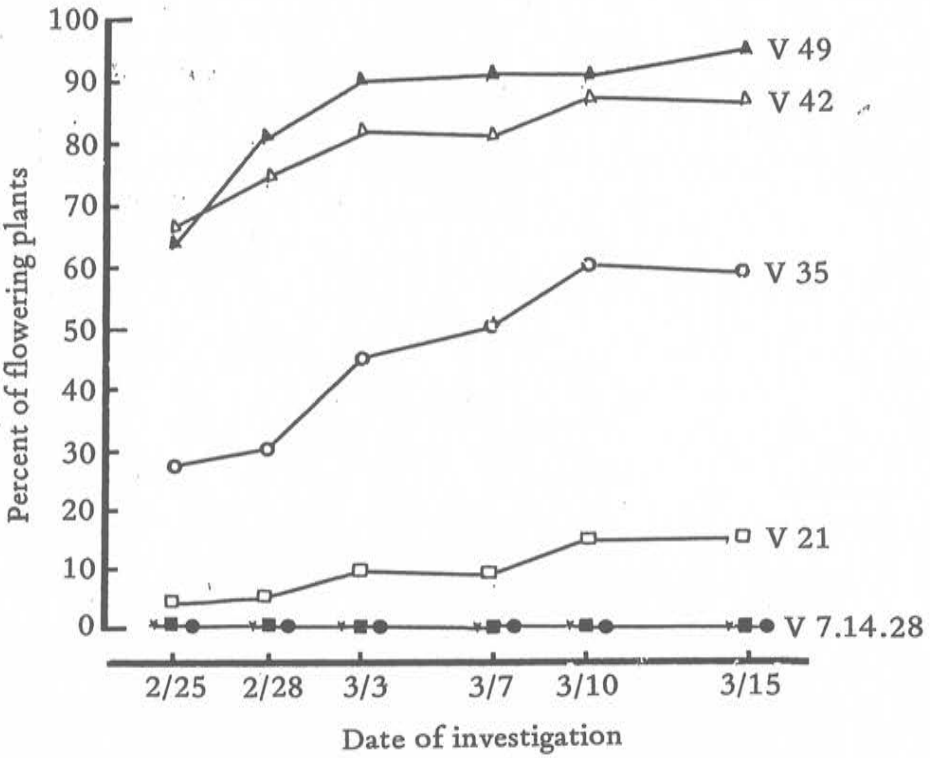


Fig. 2–4. Effect of different durations of low temperature treatment (8°C) on the flowering of kohlrabi.

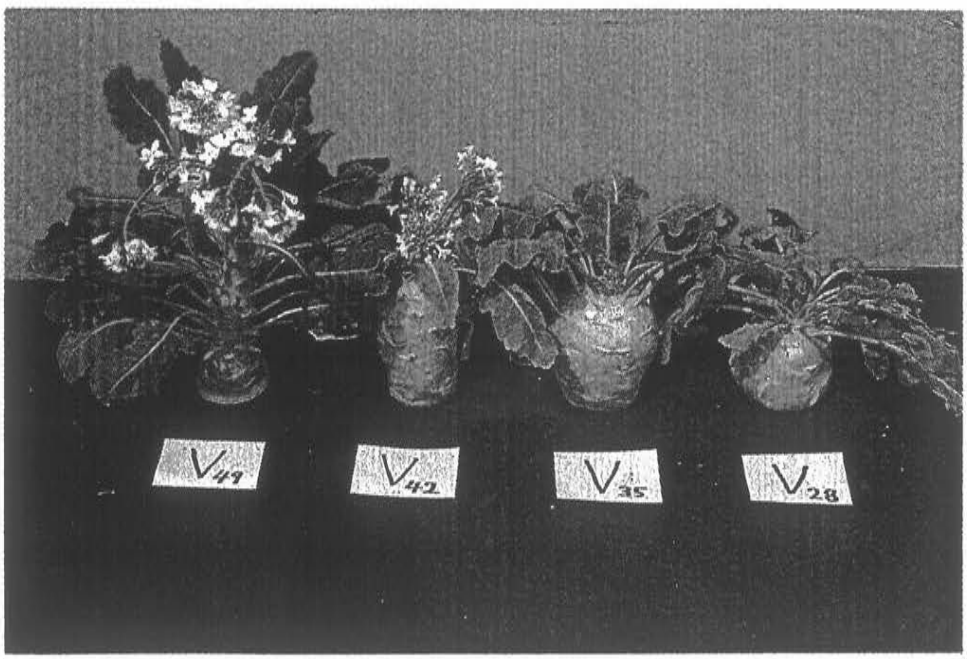


Fig. 2–5. Comparison of flowering behavior of Peking kohlrabi treated with different durations of low temperature.
V49 – vernalization for 49 days. V35 – vernalization for 35 days.
V42 – vernalization for 42 days. V28 – vernslization for 28 days.

Table 2–3. *Dry matter content (%) of the swollen stem of "Peking Late Maturing" kohlrabi after low temperature treatments*

Treatment*	%	Treatment	%	Treatment	%
I-V-0	9.32	II-V-0	7.45	III-V-0	8.11
I-V-7	7.75	II-V-7	6.83	III-V-7	5.36
I-V-14	7.52	II-V-14	6.95	III-V-14	7.23
I-V-21	8.34	II-V-21	6.74	III-V-21	6.88
I-V-28	6.26	II-V-28	7.21	III-V-28	7.28
I-V-35	6.65	II-V-35	6.07	III-V-35	6.88
I-V-42	6.63	II-V-42	5.69	III-V-42	6.59
I-V-49	5.54	II-V-49	7.54	III-V-49	7.53

*I. First sampling time at the end of vernalization
II. Second sampling time at Feb. 1, 1978
III. Third sampling time at Feb. 8, 1978
V-O. Non vernalization
V-7,14.....49. days of vernalization

sugar all increased at the beginning of vernalization. However, they were decreased if plants were given longer vernalizations (I-V-35—I-V-49). The greatest increase was in the content of reducing sugar during the first four weeks of vernalization, after which the content level led off. Total carbohydrate content also remained at high levels until the fifth week when it began falling. The pattern was the same for the C/N ratio (I-V-7, I-V-35—49).

Total nitrogen tended to decrease after vernalization in plants sampled on February 1. Plants receiving longer vernalizations tended to maintain high levels of total nitrogen after vernalization. Similar changes in soluble nitrogen content also occurred after vernalization. Total and reducing sugar contents began to decrease from the third week of vernalization, and decreased at faster rates the longer the duration of vernalization (II-V-35—II-V-49). Starch content increased beginning from the third week of vernalization in contrast to total and reducing sugars. Total carbohydrate reached a peak after the first week of vernalization (II-V-7) and then declined. The C/N ratio reached a peak after the first week of vernalization (II-V-7) and then declined steeply with longer vernalizations.

Table 2–4. *Changes in carbohydrate and nitrogen contents (% dry matter) in the swollen stem of "Peking Late Maturing" Kohlrabi after low temperature treatments of different durations*[a]

Treatment	Nitrogen			Soluble sugar			Starch	Total carbohydrates	C/N	Total soluble sugar/N
	Soluble	Insoluble	Total	Reducing	Non-reducing	Total				
I-V-0	2.16	2.28	4.44	23.25	1.63	24.88	3.86	28.84	6.50	5.60
I-V-7	2.13	1.83	3.96	24.11	3.70	27.81	3.77	31.58	7.98	7.02
I-V-14	2.13	2.66	4.79	20.33	4.36	29.46	4.57	29.26	6.11	5.15
I-V-21	2.19	2.45	4.64	23.15	4.71	27.86	3.57	31.43	6.77	6.00
I-V-28	2.59	1.97	4.56	23.81	5.14	28.95	2.58	31.53	6.35	6.78
I-V-35	2.55	2.64	5.91	19.99	6.61	26.60	3.68	25.28	4.28	3.66
I-V-42	2.65	1.44	4.09	—	—	—	—	—	—	—
I-V-49	3.09	3.16	6.25	18.88	2.84	21.72	3.81	25.53	4.09	3.48
II-V-0	1.38	1.85	3.23	29.06	5.37	34.42	3.68	38.10	11.80	10.66
II-V-7	1.38	1.55	2.93	36.71	3.54	40.25	3.46	43.71	14.92	13.74
II-V-14	1.57	2.04	3.61	20.55	6.52	27.07	3.95	31.82	8.82	7.50
II-V-21	1.84	3.06	4.90	13.67	4.62	18.29	4.21	22.49	4.59	3.75
II-V-28	1.93	2.59	4.52	12.45	3.71	16.16	4.94	21.10	4.67	3.58
II-V-35	2.68	2.28	4.96	6.04	4.61	10.56	5.61	16.26	3.28	2.15
II-V-42	2.81	2.80	5.61	9.82	8.76	18.58	4.64	23.22	4.14	3.31
II-V-49	2.95	2.23	5.18	9.58	3.42	13.00	5.05	18.05	3.49	2.51
III-V-0	1.50	1.59	3.09	27.70	7.47	35.17	4.67	39.84	11.89	11.38
III-V-7	1.67	1.88	3.05	31.21	4.92	37.13	3.99	41.12	13.48	12.17
III-V-14	1.87	0.95	1.82	25.86	15.32	41.18	3.67	44.85	15.90	14.60
III-V-21	1.56	1.43	1.99	24.11	6.05	31.16	4.05	35.21	11.78	10.42
III-V-28	1.89	3.52	5.41	17.39	9.72	27.11	4.27	31.38	5.80	5.01
III-V-35	2.49	1.82	4.31	11.28	10.75	22.03	4.59	26.62	6.18	5.11
III-V-42	2.59	2.35	4.94	12.80	13.10	25.90	3.01	28.91	5.85	5.24
III-V-49	1.70	1.44	3.14	11.40	6.17	17.57	4.20	21.77	6.93	5.60

a. See Figure 2–2. for description of treatments

Total nitrogen and soluble nitrogen contents of plants harvested on February 8 had followed patterns comparable to the plants harvested on February 1. They tended to increase with increasing vernalization but generally were at lower levels in comparison with levels of plants harvested on February 1. Total sugar and reducing sugar contents also followed patterns similar to those of the February 1 sampling, with longer vernalization tending to decrease their levels, especially after five weeks of vernalization (III-V-35—III-V-49). Although starch did not change greatly, the total carbohydrate content decrease significantly from the fifth week (III-V-35) to the seventh week (III-V-49). Shorter vernalizations maintained higher C/N ratios (III-V-7—III-V-21), and longer vernalizations reduced the ratio (III-V-28—III-V-49). For those plants reaching the flowering stage, C/N and soluble sugar/N ratios tended to be low.

(3) Highland vernalization

Plants transplanted to the lowland on January 28 began to flower on February 27 and completed flowering on March 31. Plants transplanted on February 28 began to flower on March 8 and completed flowering on April 2 (Table 2–5 and Fig. 2–6). The mean seed yield per plant were 5.6g and 4.1g respectively, due to the serious soft rot that happened during seed ripening stage.

The results indicate that the Peking Late Maturing variety of kohlrabi must reach a growth stage where it has 13 expanded leaves and a stem diameter of 6mm in order to be sensitive to low temperatures. The variety requires at least 30 to 40 days of vernalization at 8°C in order to flower. Selection for breeding programs usually takes place at the site of production. Elite

Table 2–5. *Comparison of bolting and flowering dates of "Peking Late Maturing" kohlrabi vernalized at high altitudes (Fu-Shou-San farm, 2200m)*

Date of lowland sowing	Date of transplanting to highland	Date of retransplanting to lowland	Date of bolting[a]	Date of flowering[b]	Date flowering ended
9/9	11/15	1/28	2/14	2/27	3/31
9/9	11/15	2/18	3/3	3/8	4/2

a. Date when 50% of the plants had bolted

b. Date when 50% of the plants had flowered

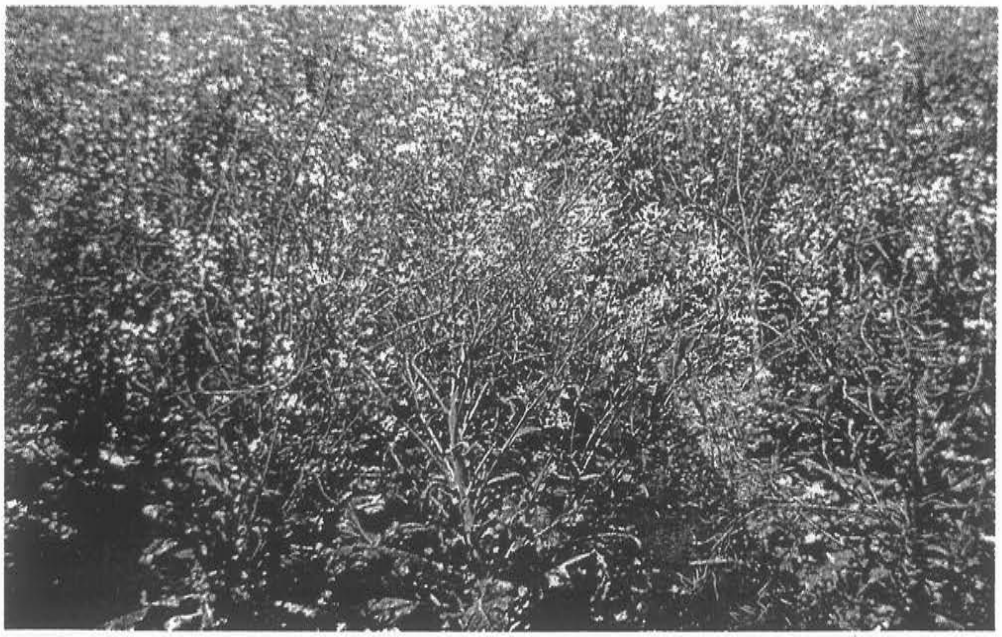


Fig. 2–6. The effect of vernalization in highland and subsequent acceleration of flowering in lowland of Peking kohlrabi.

plants can therfore be dug out and placed in an 8°C or colder environment for five to seven weeks in order to induce flowering. In Taiwan, this particular variety is usually sown from September to October and transplanted one month later. The harvest takes place in December. The selection of plants for vernalization can hence be carried out during the harvest.

Soluble nitrogen levels increased during vernalization whereas total sugar, reducing sugar and the C/N ratio increased at the beginning of vernalization and declined thereafter. The growing point began changing to a globular shape after the first week of vernalization. Total sugar, reducing sugar, total carbohydrate and the C/N ratio also reached their peak at this stage before declining. Sugar content and the C/N ratio decreased from the stages where the growing point was globular to flower bud formation.

There are various reviews on metabolic changes which occur after vernalization (Whyte 1948; Crocker 1953). Suzuki (1972) reported that an increase in carbohydrates and total sugar contents in cabbage stems preceded flower initiation after vernalization. An increase in sugar contents of the growing points of vernalizing Chinese cabbage and mustard has also been histochemically determined (Iwasaki, 1969). Sakik (1968)

also demonstrated an increase in carbohydrate content of the stem of cauliflower during the first week of vernalization, whereas no significant changes were noted in plants grown under 20–26°C. Kimura (1961) and Shinohara (1981) also reported that there was no flower initiation when explants were grown *in vitro* without sugar. The results indicate that an increase in total sugar content and a decrease in nitrogen during flower initiation thereby increases the C/N ratio. The decrease in sugar content after flower initiation suggests that energy is consumed for flower bud formation, development and bolting. The reduction might also be due to the consumption of carbohydrates in the bolting process of the formation of structural cell walls. On the other hand, nitrogen might be utilized during plant growth, thus increasing nitrogen levels slightly during the process from flower bud formation to bolting, and also decreasing the C/N ratio (Table 2–6). The response of some species to photoperiod was quite different if photoperiods were given either before or after flower bud initiation (Dostal 1950). Flower bud differentiation is thus considered a crucial stage for the transition from the vegetative to the reproductive stage. Based on Lysenko-Whyte's phasic development theory, Shinohara (1959) classified *Brassica oleracea* (n = 9) as a "green-plant vernalization type" which divides the flowering process into two phases. The thermophase is the first phase, and the photophase is the second phase. The results indicate that the first phase is from vernalization to flower bud formation with its increase in sugar level and C/N ratio, whereas the second phase is from flower bud formation to bolting with a decreasing sugar level nitrogen absorption and a decrease in the C/N ratio.

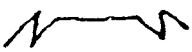
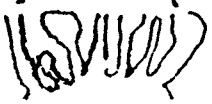
D. Summary

The experiments were carried out to investigate the conditions and development of kohlrabi flowering and its associated carbohydrate and nitrogen metabolism in relation to low temperature treatments.

Peking Late Maturing Kohlrabi is a "green-plant vernalization type". Seedling sensitivity to low temperature treatments occurs at the stage where the seedling has at least 13 expanded leaves and a stem diameter greater than 6mm. The results show that a temperature of 8°C for at least one month is required.

For the purpose of selection, mature kohlrabi was exposed to 8°C under total darkness for six weeks to induce complete flowering.

Table 2–6. *The relationship between flower development, C/N and total soluble sugar/N ratio of kohlrabi*

Treatment	Status of flower development	C/N	Total soluble sugar/N
II-V-0		11.80	10.66
II-V-7		14.92	13.74
II-V-14		8.81	7.50
II-V-21		4.59	3.75
II-V-28		4.67	3.58
II-V-35		3.28	2.15
II-V-42		4.14	3.31
II-V-49		3.49	2.51
III-V-0		11.89	11.38
III-V-7		13.48	12.17
III-V-14		15.90	14.60
III-V-21		11.76	10.42
III-V-28		5.80	5.01
III-V-35		6.18	5.11
III-V-42		5.85	5.24
III-V-49		6.93	5.60

The following metabolic changes in the swollen stems were associated with flower bud differentiation:

a. Carbohydrates increased while total nitrogen content decreased during the first week of treatment. The sugar content of the swollen stems increased rapidly in the early stages of the low temperature treatment.
b. Five week of low temperature treatments (8°C) led to flower initiation after exposure. Sugar content reached a peak before the time of flower initiation. The C/N ratio was also at a high level at this time. Longer low temperature treatments led to more floral organs, greater sugar consumption and an increase in nitrogen contents.
c. The experiment showed that longer low temperature treatments not only accelerated the development of floral organs, but also promoted perfect flowering. When the axillary flower bud and sepal developed, the sugar content decreased. The plants which bolted consumed more sugars, resulting in a lower C/N ratio. Those plants which received shorter low temperature treatments maintained high sugar content and C/N ratio but did not develop flowers, or flowered imperfectly.
d. It can be concluded that during the period from the low temperature treatment to flower initiation, increasing total sugar and decreasing nitrogen compounds form a high C/N ratio, while in the stage from flower initiation to bolting, a low C/N ratio results from a decrease in sugar content and an increase in the content of nitrogen compounds.

In subtropical Taiwan, the low temperatures required for seed production of Peking Late Maturing kohlrabi do not exist in the lowlands. It is possible, however, to expose plants at high altitudes (200m above sea level) for a period of vernalization, after which flower development and seed production can be conducted in the warm and dry lowlands.

2. *Vernalization and flower development in seed vernalization type of radish**

A. Introduction

Radish var. Ta-Mei-Fua is one of the important cultivars planted during the autumn and spring cropping seasons in Taiwan. When this crop is planted in northern Taiwan or at high altitudes, low temperatures cause premature bolting, reducing both yields and quality for fresh market production. Seed production, on the other hand, suffers from insufficiently low temperatures to induce bolting and flowering of selected seed mother plants. Therefore, methods for preventing premature bolting of an economic crop in early or late winter and artificially inducing flowering of the seed mother plant for breeding work need to be devised.

Radish is a seed vernalization type crop. A number of studies have indicated that low temperature treatments on germinating seeds are effective in accelerating flowering in radish. Yamato (1932) showed a relationship between low temperature and the flowering response of a germinating radish seed. Eguchi (1944) demonstrated that radish flowering was promoted when germinating seeds were exposed to 5°C temperatures for 15–20 days. Kagawa (1955) showed that germinating seeds treated with low temperatures and sufficient light had a better flowering response than seeds treated with low temperatures under total darkness. Sugiyama (1942) showed differences in the flowering habits of several radish varieties and used these differences to classify the radishes. Shinohara (1959) examined the genecological and population genetic aspects of phasic development and classified the climatypes of radish. While these researches on flowering physiology and morphogenesis of radish may also be applicable to the tropics and subtropics, no methods have been devised to promote flowering of selected seed mother plants under warm temperature conditions.

Seed mother plants were therefore selected for experiments to evaluate radish flowering behavior. All selected radish plants were exposed to low temperature conditions. Results from preliminary experiments indicated that radish roots appeared to vernalize efficiently when packed in transparent plastic bags and exposed to light and longer low temperature treatments. The experiment investigated the morphological changes in the shoot apex and changes in nitrogen and carbohydrate compounds in the root during flower development. The

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morphological and histochemical changes of the mature root of seed-vernalization type were also studied.

B. Materials and Methods

Radish var. Ta-Mei-Fua was sown on October 17, 1980 and harvested on November 25, 1980. Plants were selected for the experiment at harvest according to size and uniformity. The leaf blades of all selected plants were cut to 1/3 height, and then 2–3 plants were packed together in 0.02mm transparent plastic bags for treatments at 9 ± 1°C and 40 candles of artificial light. Each week, 25 plants were moved from the cold room. Twenty of these were transplanted to the field for observations on flowering response and the remaining five plants were used for analysis. This work continued every week for four weeks. A check plot was maintained in the field. On January 5, 1981, individual plants from each treatment were taken as samples. For all samples the shoot tip was fixed in a solution of formalin, alcohol and acetic acid (1:8:1). After dehydration and paraffin embedment, the tissue samples were sliced into 12–15mm sections and stained with Ehrlich's Hematoxylin for anatomical observation. Root parts were dried in an oven at 60°C for nitrogen and carbohydrate analysis. Nitrogen was analyzed by the Kieldahl method. Soluble sugar and starch were analyzed by the Anthrone method and reducing sugar by the Somogi method. A flow chart of the experimental design is shown in Figure 3–1.

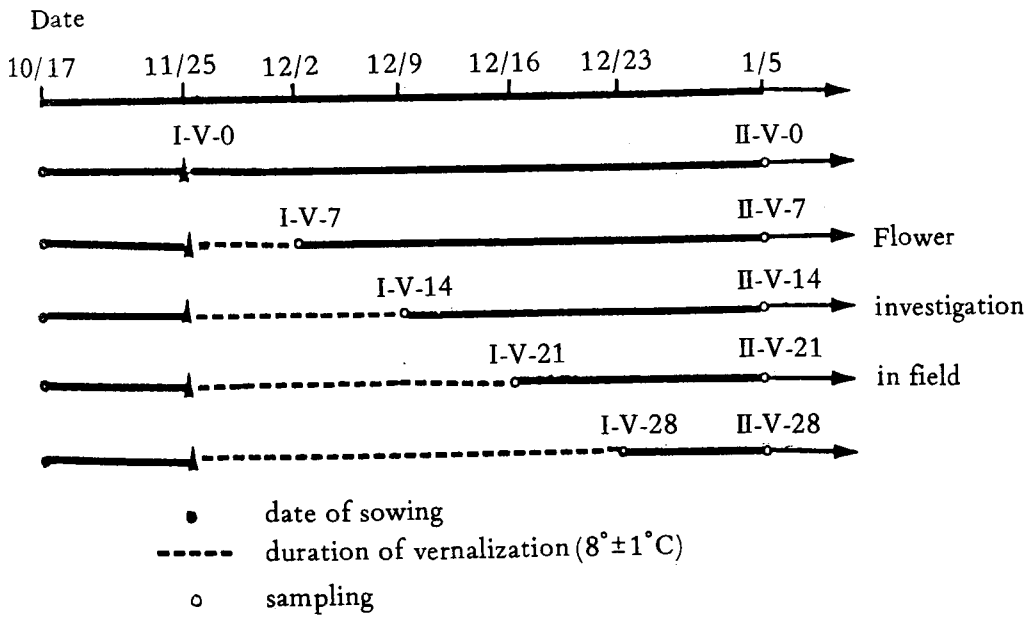


Fig. 3–1. Experimental plan for vernalization of Ta-Mei-Fua radish.

C. Results and Discussion

Changes in the dry matter content of the radish root after being exposed to low temperature treatments and transplanted to the field are shown in Table 3–1. Although new leaves continued to grow during the low temperature treatment, dry matter of the treated plots was less than that of the check, probably due to insufficient light exposure and lack of nutrient supply. Dry matter accumulation increased, however, after transplanting to the field.

Table 3–1. *Dry matter (%) of radish root after vernalization*

Treatment*	%	Treatment	%
I-V-0	5.9	II-V-0	5.6
I-V-7	5.1	II-V-7	5.8
I-V-14	5.3	II-V-14	6.4
I-V-21	5.3	II-V-21	6.4
I-V-28	5.5	II-V-28	6.2

*I. First sampling time at end of vernalization
II. Second sampling time at Jan. 5, 1981
V-O. Non vernalization
V-7, 14, ...28. days of vernalization

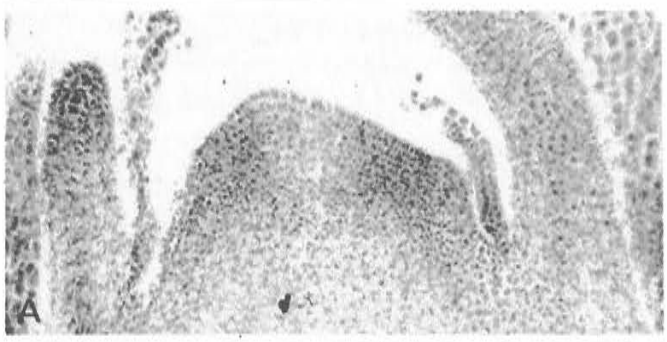
Table 3–2 and Figure 3–2 show changes in the tunica layer of the shoot apex after low temperature treatments. The shoot apex growth of the check plants appeared flat and contained 4–5 tunica layers (I-V-0). After one week of low temperature treatments the shoot apex appeared oblong and still maintained 4–5 tunica layers (I-V-7). The height and thickness of the shoot apex increased after two weeks of low temperature treatments. The number of tunica layers increased to 5–6, and more cell division activity appeared on the growing point with many new cells accumulating on the upper part of the corpus layer. The tunica layers then entered a transitional phase (I-V-14, I-V-21). The shoot apex appeared taller and tunica layers decreased from the original 5–6 layers to 3 layers after 3–4 weeks of low temperature treatments. At the same time, the cells of the corpus layer assumed a more swollen shape (I-V-21, I-V-28). Flower primordia were initiated on the shoot apex of one plant after four weeks of low temperature treatments (I-V-28). Plants transplanted to the field on January 5, 1981 after one

Table 3–2. *Changes in tunica layers of the shoot apex after low temperature treatments*

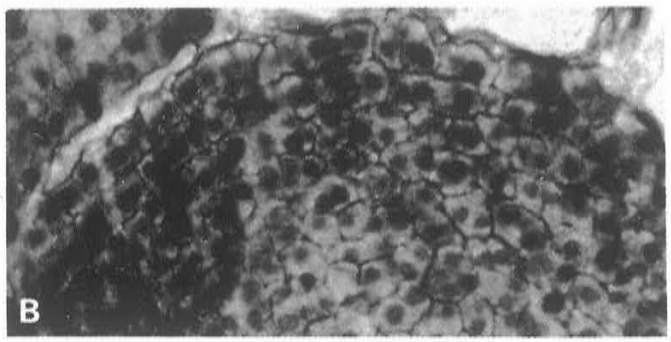
Treatment	No. of plants examined	No. of tunica layers			Plants of floral primordia initated
		4–5	5–6	3	
I-V-0	5	5			0
I-V-7	5	4	1		0
I-V-14	5	3	2		0
I-V-21	5	1	3	1	0
I-V-28	5		3	2	1

week or two weeks of low temperature treatments showed some flower primodium differentiation, but the plants exposed to four weeks of low temperatures were already bolting (II-V-28). Figure 3–3 illustrates the effects of different durations of low temperature treatments on flowering behavior. The plants exposed to low temperatures for three weeks were the first to flower on February 8. All of the low temperature treatments had at least some plants that flowered, while the non-treated check had no flowering plants. The plants exposed to low temperatures for three weeks demonstrated the fastest flowering rate.

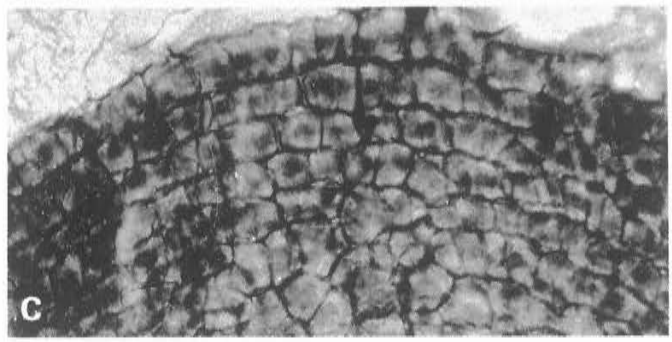
Table 3–3 shows the changes in nitrogen and carbohydrate contents of radish roots. Total nitrogen did not change significantly, but soluble nitrogen tended to increase with a peak after the first week of low temperature treatment. Accordingly, insoluble nitrogen tended to decrease, reaching a nadir after the first week. Total carbohydrates, total sugar, non-reducing and reducing sugar increased up until the third week of low temperature treatments after which they decreased. C/N and total sugar/N ratios were at a maximum after two weeks of low temperature treatments. Starch contents tended to decrease. After the plants were harvested for observation on January 5, 1981, the soluble nitrogen and sugar contents tended to be less than the levels during vernalization. The non-reducing sugar, total sugar and total carbohydrate contents were highest in the plants which had been exposed for three weeks of low temperatures (II-V-21). Reducing sugar, non-reducing sugar and total sugar contents decreased when plants began to bolt after four weeks of low temperature treatment. Soluble nitrogen contents were the higher the longer the low temperature treatments. Total nitrogen contents of all temperature treatments were lower than that of the check plot except for the four-week treatment. This in-



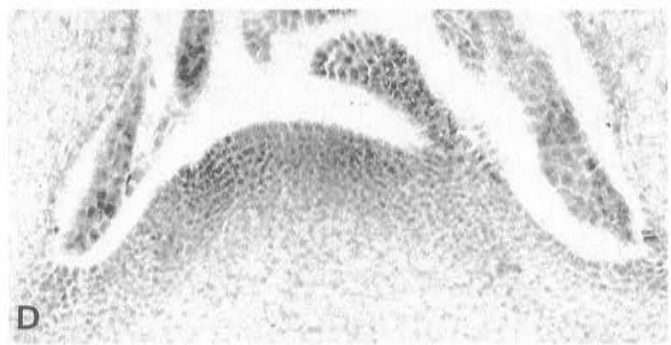
A. Non-treated (I-V-0) – Vegetative adult. 4–5 Tunica layers were observed.



B. One week treatment (I-V-7) – Vegetative stage is maintained; 4–5 tunica layers were observed. The height of the apex increased and the top grew ellipsoid.

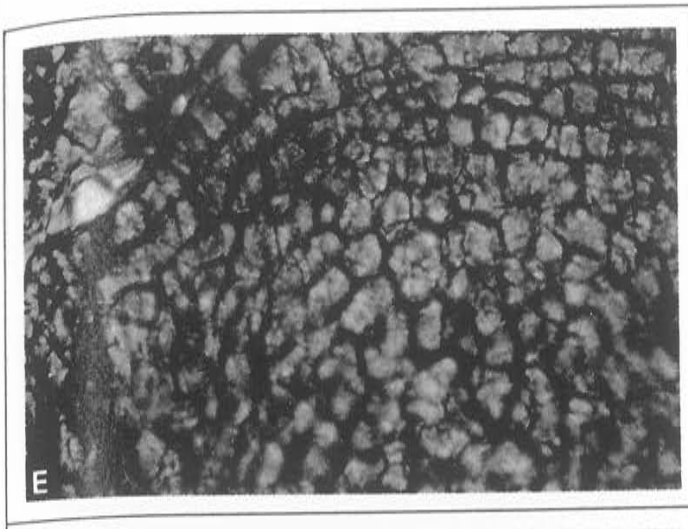


C. Two weeks treatment (I-V-14) – The apex gradually grew taller and thicker. 5 tunica layers were observed. Cell division increased on the upper part of the corpus.

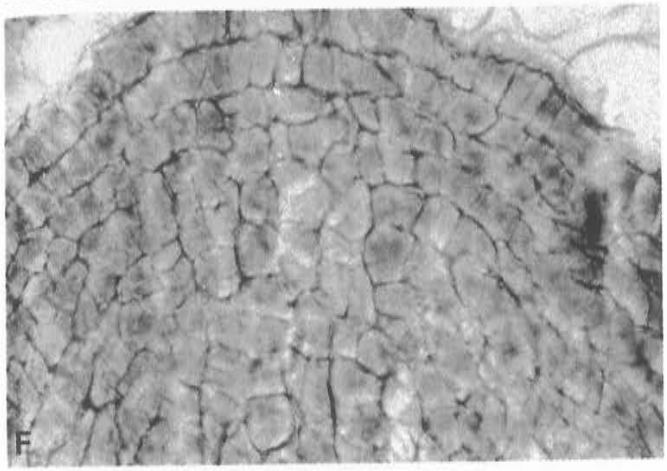


D. Three weeks treatment (I-V-21) – 6 tunica layers were observed.

Fig. 3–2. Floral development of radish root treated with different durations of low temperatures. See Figure 3–1 for reference to the experimental plan.



E. Four-weeks treatment (I-V-28) – The tunica layers decreased to 3; the shoot apex was more elongated. The first floral primordium was initiated at the inner part of the apex.



F. Observation on January 5 of the one week treatment (II-V-7). The tunica layers decreased to 3 and many new cells accumulated on the uppers part of the corpus.

G. Observation on January 5 of the four week treatment (II-V-28) – Flower bud and sepal were formed.

Fig. 3–2. Floral development of radish root treated with different durations of low temperatures. See Figure 3–1 for reference to the experimental plan.

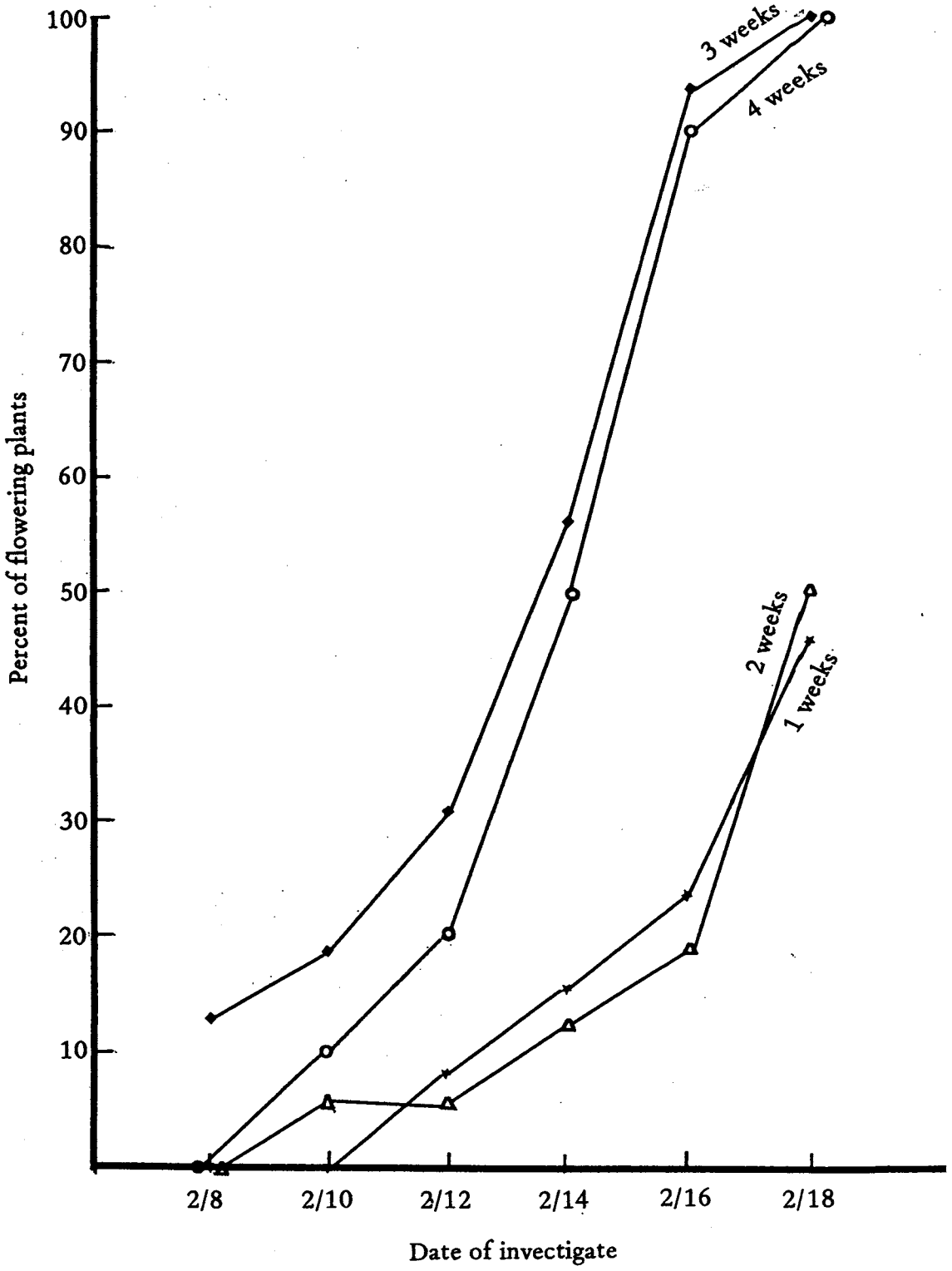


Fig. 3–3. Effect of different duration of low temperature treatment (9°±1°C) on the flower behevior of Ta-Mei-Fua radish.

Table 3—3. *Changes in carbohydrate and nitrogen contents (% dry matter in radish root exposed to low temperatures for different durations*

Treatment	Nitrogen			Soluble sugar			Starch	Total carbo-hydrates	C/N	Total soluble sugar/N
	Soluble	Insoluble	Total	Reducing	Non-reducing	Total				
I-V-0	1.24	1.49	2.73	21.3	14.1	35.4	7.4	42.8	15.7	13.0
I-V-7	1.64	0.99	2.63	24.3	18.2	42.5	4.6	47.1	17.9	16.2
I-V-14	1.39	1.17	2.56	24.4	18.7	43.1	3.8	46.9	18.3	16.8
I-V-21	1.33	1.39	2.72	24.6	19.5	44.1	3.4	47.5	17.1	16.2
I-V-28	1.34	1.29	2.63	23.3	17.5	40.8	2.0	42.8	16.3	15.5
II-V-0	0.98	2.14	3.12	19.5	5.9	25.4	2.2	27.6	8.8	8.1
II-V-7	1.10	1.50	2.60	17.4	9.5	26.9	4.6	31.5	12.1	10.3
II-V-14	1.18	1.71	2.89	19.9	13.2	33.1	4.8	37.9	13.1	11.4
II-V-21	1.22	1.45	2.67	12.3	25.2	37.5	5.0	42.5	15.2	14.0
II-V-28	1.46	1.71	3.17	13.4	14.5	27.9	6 4	34.3	10.8	8.8

dicates that plants begin to absorb nitrogen when bolting, and thus lower both C/N and total sugar/N ratios.

Wang (1969) observed that the number of tunica layers on the shoot apex of To-pe-tsai (*B. chinensis* L.) increased from 4–5 to 5–6 layers when the plant changed from the vegetative phase to the reproductive phase. This number rapidly decreased to 3 layers before the flower primordium was initiated. Radish demonstrated the same phenomenon, with increasing sugar contents before flower bud differentiation. The sugar content (Table 3–3) continued to increase as the tunica layers increased from 4–5 layers (Table 3–2, I-V-4) to 5–6 layers (Table 3–2, I-V-21) and then gradually decreased (Table 3–2, I-V-28) as the tunica layers decreased to three layers. With the appearance of the flower primordium, the sugar content decreased even more (Table 3–2, II-V-28), especially when plants began to bolt. This indicates that the cell division stage consumes more sugar, and that the increase and subsequent decrease in sugar contents were related not only to flower bud formation but to flower development and bolting as well.

The decrease in starch contents and increase in reducing sugar, non-reducing sugar and total sugar contents during flower bud differentiation were the same as reported by Suzuki (1972) for cabbage. The results were similar also to results from previous research on the efficiency of low temperature treatments on mature kohlrabi, and showed that flower bud formation in radish increased sugar contents during vernalization. Sugar contents gradually decreased from flower primordium development to bolting. At the same time, the C/N ratio tended to decrease due to the absorption of nitrogen by the plant during bolting. Therefore, the low temperature response of flower bud differentiation was an increase in sugar content, with the total sugar/N ratio reaching a peak just before flower bud differentiation. When the plants passed into the reproductive or bolting stage, sugar contents decreased and nitrogen was absorbed by the plant, decreasing both the total sugar/N and C/N ratios. This pattern of metabolic changes during flower development in radish was the same as demonstrated in previous research on kohlrabi.

D. Summary

Seed vernalization type of radish was used to find a method to promote flowering in slow bolting radish for breeding in the tropics. The relationship between morphological changes of the shoot apex and metabolic changes in nitrogen carbohydrates compounds during flower

development was also investigated.

Results indicated that mature Ta-Mei-Fua radish will flower when defoliated of top parts and the root exposed to 9 ± 1°C temperature and 12 hr day lengths for three weeks.

The number of tunica layers on the shoot apex increased from 4–5 layers to 5–6 layers when mature plants were exposed to three weeks of low temperature treatments. The number of layers decreased rapidly to three layers when mature plants transited from the generative stage to the reproductive stage. At the same time, cell shapes on the corpus layers changed from their original flat type to an erect swollen type. The flower primordium began to form after four weeks of low temperature treatments. Sugar content increased during low temperature treatments and was highest when cell division on the shoot apex was at a maximum and the tunica layers were increasing to 5–6 layers. Sugar content gradually decreased as the number of tunica layers decreased to three and flower primordium formation gave way to bolting. Changes in sugar contents were similar to those of green-plant vernalization type crops when treated with low temperatures.

3. Comparison of effectiveness of flower induction between green-plant and seed-vernalization types

A. Introduction

Mature plants of kohlrabi (green-plant vernalization type) and radish (seed-vernalization type) have demonstrated flowering after low temperature treatments. Flower formation in these plants was promoted by an increase in their sugar contents. In the previous two experiments, the behavior of the sugar contents of these plants during flower induction was compared after plants were given low temperature treatments. The seed of these two types of plants were also exposed to low temperatures to compare the activities of nitrogen and carbohydrate compounds and the changes resulting from such treatments.

B. Materials and Methods

Radish (var. Ta-Mei-Fua) and kohlrabi (var. Peking) seeds were pre-germinated at 20°C for 24 hours, and then placed in a cool room at 4 ± 1°C for 1, 2, 3, and 4 weeks. Untreated seeds were used as a check. After each treatment, half of the seeds from each group were then planted in 4-inch pots at 20°C for observation of flowering behaviour. The remaining seeds were used for analysis of nitrogen and carbohydrate contents in accordance with previous methods of analysis. The experimental design is shown in Figure 4–1.

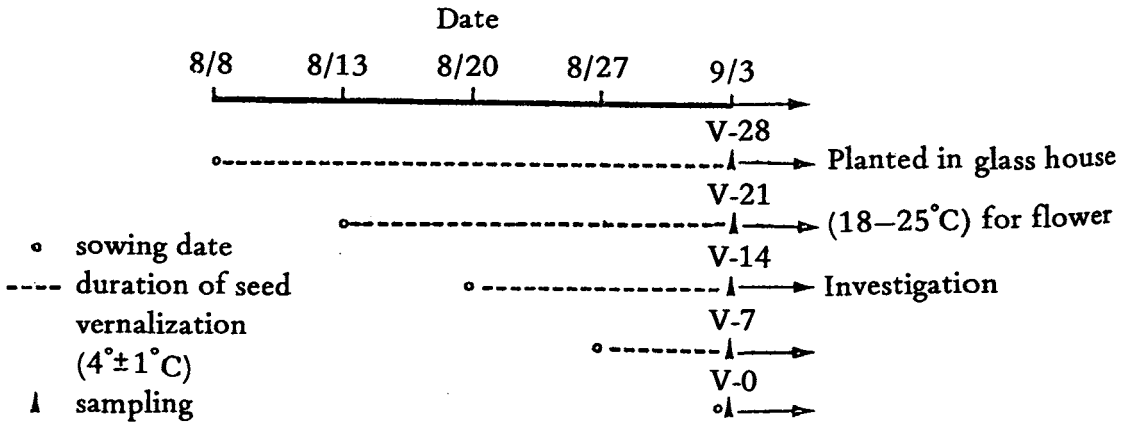


Fig. 4–1. Experimental plan on seed vernalization of Ta-Mei-Fua radish and Peking kohlrabi.

Presented at the Joint Agricultural Seminar of the National Chung-Hsing University and California State University, Fresno. July, 1982 and Published at J. Chinese Soc. Hort. Sci. 29(4): 279–290, 1983.

C. Results and Discussion

The flowering behaviour of radish (var. Ta-Mei-Fua) after seed vernalization is shown in Figure 4–2. All of the plots exposed to low temperature began to flower 28 days after sowing, and flowering rates on November 3 were 50%, 70%, 100% and 96% for the 1, 2, 3 and 4-week treatments respectively. The check did not flower. The fastest flowering rate was from the plot exposed to low temperatures for three weeks. The changes in nitrogen and carbohydrate contents of the seeds treated with low temperatures are shown in Figure 4–3 and Figure 4–4. Reducing sugar, total sugar and total carbohydrate contents were highest after the first week of treatment and then gradually decreased. Starch content tended to be stable even with lengthening low temperature conditions while non-reducing sugar decreased noticeably. Although total nitrogen did not change under low temperatures, soluble nitrogen tended to increase with longer durations of low temperatures.

Kohlrabi (var. Peking) seeds did not flower regardless of treatment, indicating that kohlrabi is a green-plant vernalization type. Total nitrogen, soluble nitrogen and non-soluble nitrogen contents did not change during any low temperature treatments. There also were no differences in the behavior of the sugar and starch contents of the check plot and the low temperature treated plots.

Suzuki (1972) indicated that for seed-vernalization type of turnip, the highest level of total carbohydrate, total sugar and reducing sugar contents was attained 20 days after low temperature treatment. For radish, the highest levels were attained after the first week of low temperature treatments, indicating differences between crops in their response to low temperature durations. The results might show that the longer the duration of low temperatures required to promote flowering, the more slowly these compounds reached a plateau. The behavior of non-reducing sugar, starch and nitrogen in turnip seed was similar to that observed in radish.

Mikhailova (1949) indicated that sugar contents did not increase in the plant vernalization type of cabbage seeds after 80 days of low temperature treatments, agreeing with results obtained in this experiment with kohlrabi seed.

The experiment showed that flower induction of seed-vernalization type plants was the result of an increase in sugar content during low temperature treatments. The lack of a similar increase in the green-plant vernalization type plants resulted in no flower induction. The total sugar content of radish seeds was more than twice that of kohlrabi

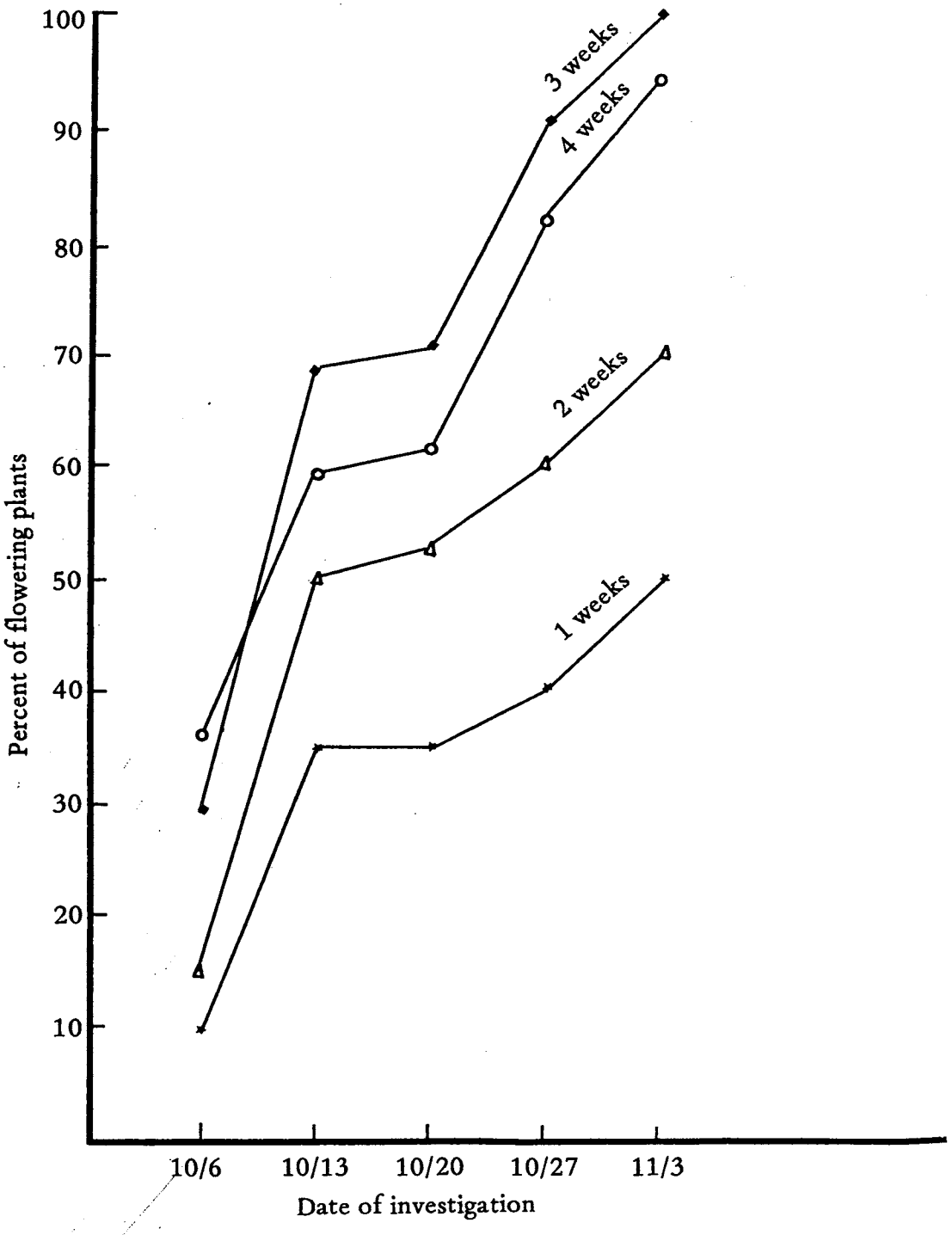


Fig. 4–2. Effect of different duration of seed vernalization (4°±1°C) on the flower behavior of Ta-Mei-Fua radish.

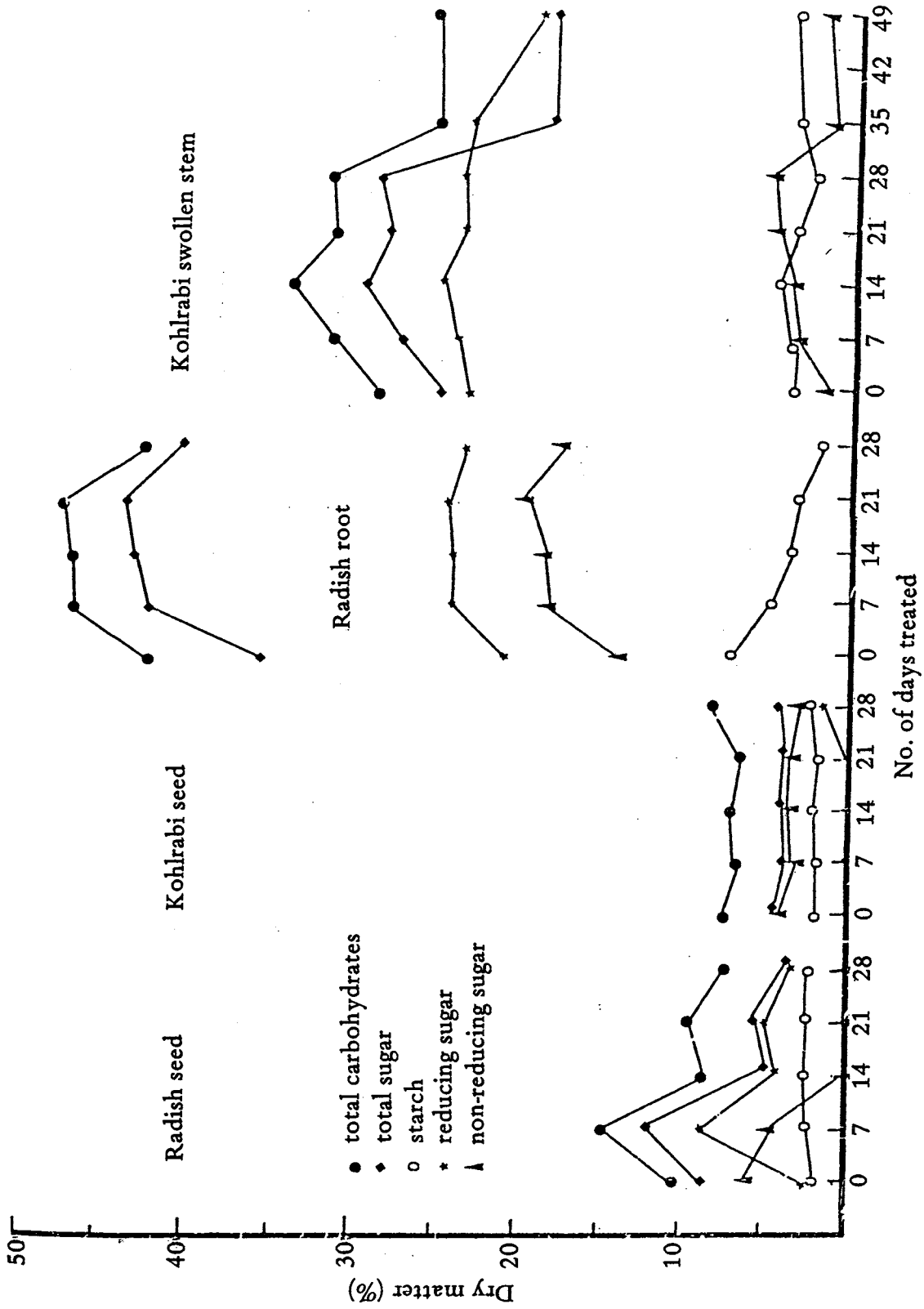


Fig. 4–3. Comparative changes in carbohydrate contents in kohlrabi and radish exposed to different durations of low temperature.

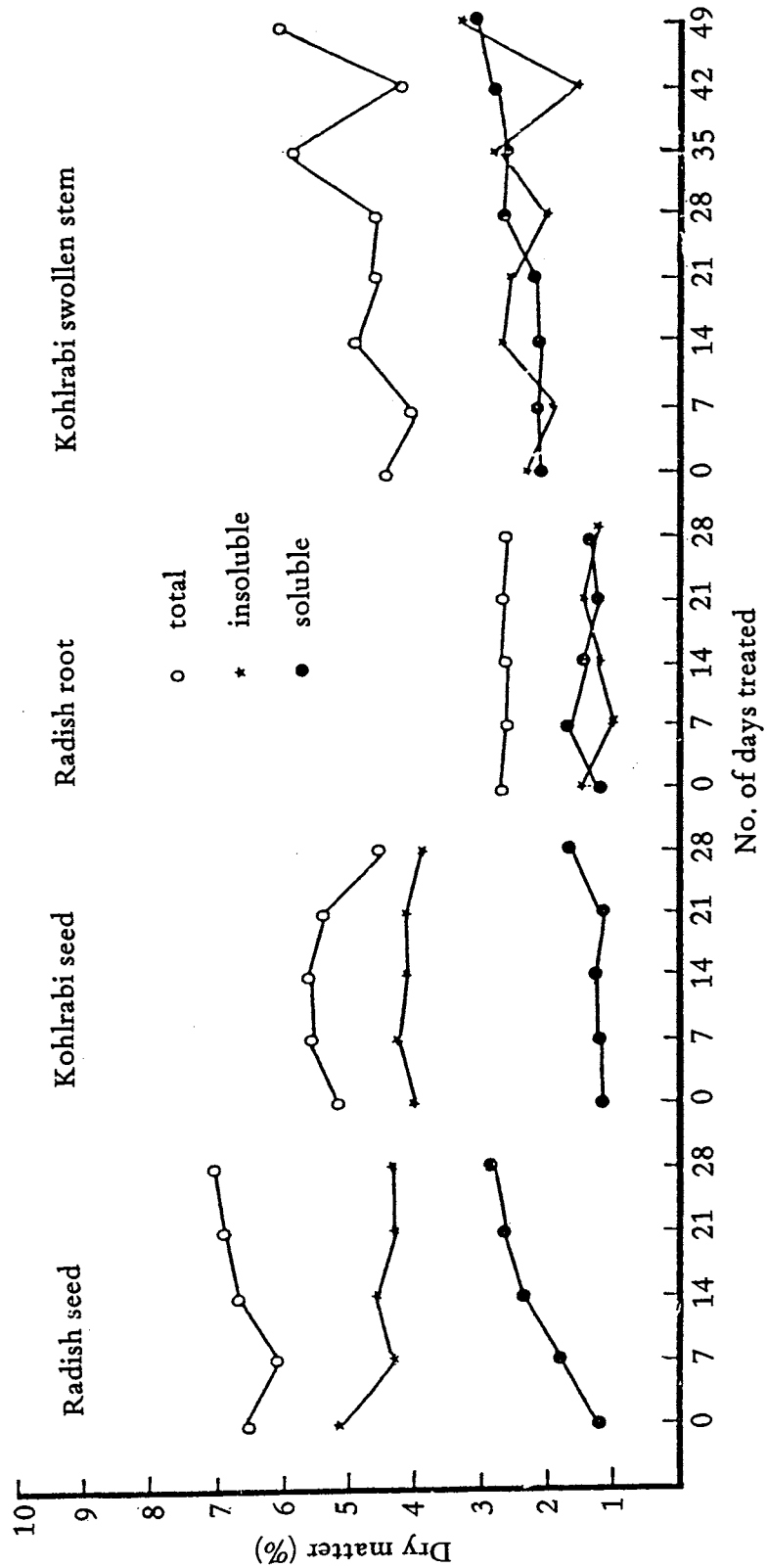

Radish seed
Kohlrabi seed
Radish root
Kohlrabi swollen stem
o total
* insoluble
● soluble
Dry matter (%)
No. of days treated
0 7 14 21 28 0 7 14 21 28 0 7 14 21 28 35 42 49
1 2 3 4 5 6 7 8 9 10

Fig. 4–4. Comparative changes in nitrogen content in kohlrabi and radish exposed to different durations of low temperature.

before vernalization. For radish, the reducing sugar content rapidly increased and non-reducing sugar content gradually decreased during low temperature treatments. There were no changes in the reducing or non-reducing sugar contents of kohlrabi. The results suggest that the hydrolysis of non-reducing sugar to reducing sugar in radish seeds under low temperature conditions might be a key point in radish flower induction.

Mature plants of both radish and kohlrabi flowered after low temperature treatments. Radish plants flowered after one week of low temperatures and flowered completely when exposed for three weeks (Figure 3—3). Kohlrabi did not flower after four weeks of low temperatures, but flowered completely after 6—7 weeks of low temperatures (Figure 2—4).

Non-reducing sugar content showed the greatest difference in the contents of radish and kohlrabi. The non-reducing sugar content of radish was 8—9 times that of kohlrabi before the low temperature treatments. Both radish and kohlrabi had increasing total carbohydrate, total sugar and non-reducing sugar contents at the beginning of the low temperature treatments and declining contents thereafter. Starch content decreased slightly in both radish and kohlrabi during low temperature treatments. Although changes in total nitrogen content were slight, soluble nitrogen increased slightly during vernalization. The rise in soluble nitrogen content was earlier in radish than in kohlrabi. This indicates that mature plants of both green-plant vernalization and seed-vernalization types contained sufficient level of mobile sugar even before low temperature treatments. Sugar content also decreased during bolting. Changes in nitrogen and carbohydrate metabolism for mature plant vernalization were also similar for both vernalization types. Miller (1929) and Boswell (1929) showed that sugar and carbohydrate contents tended to increase in cabbage which had over-wintered. Similar

Table 4—1. *Dry matter (%) after seed vernalization*

Treatment	Radish	Kohlrabi
V-0	56.5	54.2
V-7	31.8	45.6
V-14	26.4	41.5
V-21	24.4	37.5
V-28	17.3	32.5

results were found with wheat (Gregory 1936 and 1938). Suzuki (1972) showed that non-reducing sugar and total carbohydrate contents of the stem and leaves of cabbage seedlings were highest after 30 days of low temperature treatments. The present experiment determined that total sugar and non-reducing sugar contents were highest after 28 days and 21 days of low temperature treatments for kohlrabi (var. Peking) and radish (var. Ta-Mei-Fua) respectively. The sensitivity for the green plant of Chinese cabbage was determined by the genetic background and its thermostage. (Yamasaki, 1956 and 1962). Green-plant vernalization types would have patterns of flower formation and development identical to those of seed-vernalization types if they reach a certain stage of maturity.

Comparing nitrogen and carbohydrate contents of kohlrabi seeds and mature plants, total sugar content of the seeds was found to be only about 1/5 that found in mature plants, and almost no reducing sugar was found in seeds before low temperature treatments. Reducing sugar, non-reducing sugar, total sugar and soluble nitrogen contents of the mature plants tended to increase under low temperature treatments. On the other hand, no changes were noted for seeds undergoing low temperature treatment. These results suggest that the level of sugar content was lower in seeds of green-plant vernalization types and that plants of this vernalization type must grow a certain stage in which sugars reach a certain level for attainment of responsiveness to cold.

Comparative changes in seeds and mature plants of radish, mature radish plants had higher nitrogen and carbohydrate contents before low temperature treatments than did seeds. Although the sugar content in seeds was lower, a certain level was still present, suggesting that this sugar content may be a basic condition for low temperature sensitivity. Reducing sugar, total sugar, total carbohydrate and soluble nitrogen contents all tended to increase in both seeds and mature plants during low temperature treatments. Total nitrogen, however, did not change significantly in either. Non-reducing sugar content increased and starch content decreased in mature plants exposed to low temperatures. In seeds, however, the opposite was the case, with non-reducing sugar tending to decrease and starch tending to be stable during low temperature treatments. The changes noted in radish seeds were the same as identified by Suzuki (1972) in turnip seeds. A decrease in non-reducing sugar and an increase in reducing sugar of seeds, or a decrease in starch and an increase in sugar of mature plants during low temperature treatments might be the cause of flower formation in seed vernalization type plants.

The results suggest that effective or non-effective seed vernalization is determined by changes in sugar contents, especially the canges in reducing sugar and total sugar contents under low temperature conditions. Reducing sugar could possibly originate from the non-reducing sugars of the seed, but what kinds of reducing or non-reducing sugars are contained in the seed is still unknown. In general, the sugars are reversible through sucrose synthesis or degradation according to the reaction:

$$\text{glucose-1-phosphate} + \text{fructose} \xrightleftharpoons{\text{sucrose phosphorylase}} \text{sucrose} + \text{phosphoric acid}$$

Sucrose phosphorylase catalyzes both reactions, and non-reducing sugars can be synthesized or degraded in seed-vernalization type plants depending on low temperature conditions. On the contrary, level of sugars connot be degraded in green-plant vernalization types by seed vernalization. The sugar contents of mature plants was high, and total sugar, reducing sugar and non-reducing sugar contents increased during low temperature treatments. Starch content noticeably decreased in radish at the same time. Starch is made up of two components, amylose and amylopectin. Amylose is more soluble in water and is possibly synthesized or degraded according to the reaction:

$$\text{glucose-1-phosphate} \xrightleftharpoons{\text{starch phosphorylase}} \text{amylose} + H_2PO_4$$

Starch thus might be degraded to reducing sugars or non-reducing sugars in mature plants depending on low temperature treatments.

D. Summary

Effective seed vernalization in seed vernalization plants was accompanied by an increase in sugar content during low temperature treatments. An ineffective seed vernalization of green-plant vernalization plants showed no such sugar increase during vernalization. Green-plant vernalization plants showed the same changes in sugar content as seed vernalization types, but only after the plant had grown to a certain stage at which it was responsive to low temperature treatments.

Seed-vernalization type (plants having a facultative requirement) contained higher levels of sugar in the seed while green-plant vernalization types (plants haveing an obligate requirement) contained lower

levels of sugar only. A low temperature response from these crops was due to a gradual increase in level of sugar during the growing process. Mautre plants of seed-vernalization and green-plant vernalization types contain higher levels of sugars, making them more responsive to low temperature conditions.

Ⅲ. Effect of day and night temperature and of high temperature on devernalization

A. Introduction

High temperature following vernalization has been reported to erase the effect of low temperature treatment (Gregory and Purvis 1945, 1952; Lang and Melchers 1947; Suzuki 1980). Gregory and Purvis (1952) found that vernalization in winter rye could be reversed if followed immediately by exposure to temperature above 25°C–35°C. Suzuki (1980) investigated the effect of day-night temperature variation on the flowering of radish and showed that when plants were exposed to 4°C at night and 30°C during the day, the effect of vernalization was erased by the high daytime temperature.

In temperate countries, plastic is used as a mulching material to prevent premature bolting during the winter season. Seyama (1981) indicated that radish planted with mulching had a maximum day temperature higher than that planted under natural field conditions. The higher day temperature could nullify the effect of night vernalization. In the tropics, the degree and duration of low temperature conditions are not enough to slow down bolting in flowering varieties. However, it is not known whether variation in day-night temperature, especially higher daytime temperature, is the factor that prevents bolting, or the effect of high temperature causing devernalization following a continuous period of vernalization. Devernalization constraints may be more serious in the tropics than in temperate areas. This study addresses these problems.

B. Material and Methods

Radish var. Tai-Mei-Fua was used as the experimental material and the following two experiments were carried out from September 1982 to April 1983:

(1) Effect of alternating high day/low night temperature on bolting during vernalization

The first group of seeds was sown in Jiffy-7 (peat pellet) on September 14, 1982. The group was divided into three parts which received the following treatments for 20 days starting on September 22: A) temperature treatment of 5°C at night (20:00 to 8:00 in a cool room) and 30.3°C in the daytime (8:00 to 20:00 in the greenhouse) (Table 5–1); B) 5°C at night and

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20°C in the daytime; C) constant temperature of 5°C (check). A second group of seeds was sown on September 24, 1982 and received the same treatments for 10 days beginning on October 2. On October 12, all seedlings were planted in the field for flowering investigation (Figure 5–1).

Table 5–1. *Day temperature in the greenhouse (8:00–20:00)*

Date	9/23	9/24	9/25	9/26	9/27	9/28	9/29
Maximum	31	31	33	32.5	32	33	32
Minimum	27.5	28	28	28	29	29	28
Mean	29.3	30	30.5	30.3	30.5	31	30
Date	9/30	10/1	10/2	10/3	10/4	10/5	10/6
Maximum	32	33	33	31.5	32.5	33.0	31.5
Minimum	28	29	29	28.5	29	29	28
Mean	30	31	31	30	20.8	31	29.8
Date	10/7	10/8	10/9	10/10	10/11	10/12	mean
Maximum	33	32	32	32	32.5	31.5	32.3
Minimum	29	28	29	28	28	27	28.3
Mean	30.5	30	30.5	30	30.3	29.3	30.3

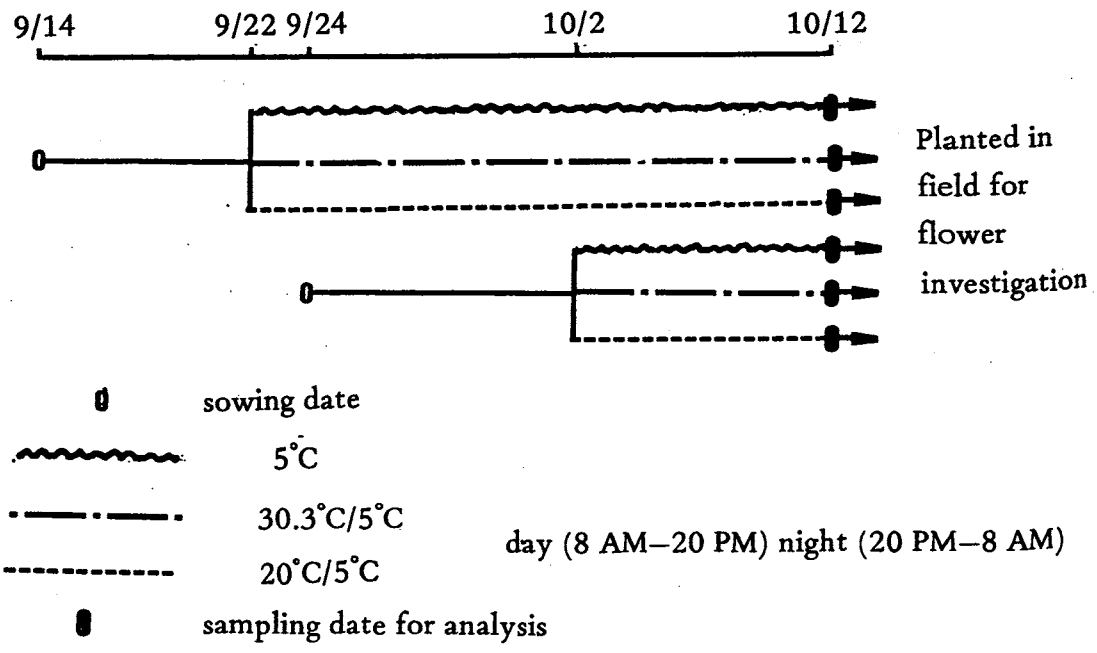

Fig. 5–1. Plan for effect of alternating day/night temperature on flowering of radish seedling.

(2) Effect of high temperature following an uninterrupted period of vernalization on bolting

Two groups of seeds were sown in Jiffy-7 (peat pellet) on December 21 and December 31. Ten days after planting, they received cold treatment of 5°C for 20 and 10 days, respectively. After vernalization, each group was divided into three parts which received the following treatments for 10 days: A) 30°C; B) 20°C; C) alternating day temperature of 30°C and night temperature of 20°C. On January 29, all treated radish were planted in 4.5 inch pots and placed in the greenhouse for bolting investigation (Figure 5–2).

After high temperature treatments, samples of all plants from both experiments were taken for analysis of carbohydrate contents.

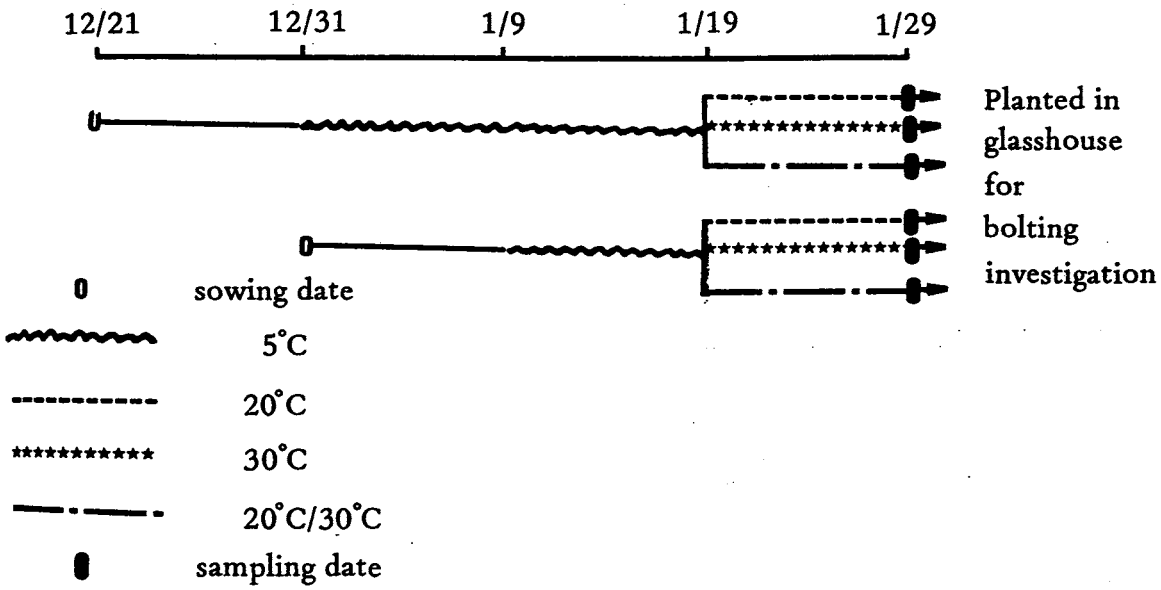

Fig. 5–2. Plan for effect of high temperature and alternating day/night temperature after an uninterrupted period of vernalization on bolting.

C. Results and Discussions

(1) Effect of alternating high day/low night temperatures on flowering

The check maintained at 5°C for 20 days showed complete vernalization whereas 5°C for 10 days was not enough to induce vernalization completely in radish. The number of flowering

plants was lower and flowering dates were later for the 10-day treatment than for the 20-day treatment. Alternating day/night temperatures of 20°C/5°C and 30.3°/5°C for 10 days did not induce flowering until the end of the experiment, wheras 2 and 1 plants flowering in alternating day/night temperature of 20°/5°C and 30.3°/5°C for 20 days respectively (Table 5–2). Apparently, high daytime temperatures of 30.3°C during vernalization could certainly erase the effects of cold night temperatures. Even a relatively high daytime temperature around 20°C during the period of vernalization can nullify the effect of vernalization.

Table 5–2. *Effect of alternating day/night temperature on flowering of radish*

Temperature (night/day)	Days of treatment	No. of plants treated	No. of flowering plants				
			11/18	11/23	11/28	12/3	12/8
5°C / 5°C	20	30	14	18	26	28	30
5°C / 30.3°C	20	30	0	0	0	0	0
5°C / 20°C	20	30	0	0	0	0	0
5°C / 5°C	10	30	0	0	2	4	8
5°C / 30.3°C	10	30	0	0	0	0	0
5°C / 20°C	10	30	0	0	0	0	0

Temperature (night/day)	Days of treatment	No. of plants treated	No. of flowering plants			
			12/13	12/18	12/23	12/28
5°C / 5°C	20	30	–	–	–	–
5°C / 30.3°C	20	30	0	0	0	1
5°C / 20°C	20	30	0	0	0	2
5°C / 5°C	10	30	8	9	9	9
5°C / 30.3°C	10	30	0	0	0	0
5°C / 20°C	10	30	0	0	0	0

Suzuki (1981) indicated that day/night temperature variation plays an important role in inducing flowering in radish. Under the condition of 30°C day/4°C night, the effect of vernalization was erased by the higher day temperature. Seyama

(1981) also showed that the bolting effect of low temperature on radish could be annulled by 30°/5°C day/night temperature treatment. He indicated that mulching could prevent premature bolting by raising temperatures. In winter rye, Gregory and Purvis (1938) showed that six weeks of cold treatment (1°C). Could induce flowering, whereas alternating temperatures at 1°C with aerobic and 20°C with anaerobic conditions for six weeks, winter rye remained unvernalized. All of these results suggest that high temperature during the day could erase the effect of low temperature during the vernalization process.

Patterns of the carbohydrate contents during vernalization are shown in Table 5–3. The sugar contents of radish treated with 5°C for 10 days and 20 days are higher compared with those grown under alternating day/night temperature treatments of 20°C/5°C and 30.3°C/5°C. Among these, seedlings treated with 5°C for 20 days had the highest non-reducing sugar, total sugar, and total carbohydrate contents, whereas treatment of 5°C for 10 days gave the highest reducing sugar contents of all treatments. The alternating of high day temperature with low night temperature, resulted in low sugar contents. Although day temperatures of 30.3°C and 20°C for 10 or 20 days did not show much difference in sugar contents, the non-reducing sugar content of the 20°C day-temperature treatment was a little higher than that of the 30.3°C treatment.

Sugar content in the alternating day/night temperature treatment did not increase during the treatment period. In

Table 5–3. *Carbohydrate contents (% dry matter) of radishs seedlings under various day/night temperature treatments*

Temperature (night/day)	Days of treatment	Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrate
5°C / 5°C	20	1.77	3.66	6.43	6.33	12.76
5°C / 30.3°C	20	0.98	1.06	2.04	5.19	7.23
5°C / 20°C	20	0.98	1.48	2.46	7.05	9.51
5°C / 5°C	10	2.52	0.64	3.16	4.87	8.03
5°C / 30.3°C	10	0.94	1.07	2.01	5.30	7.31
5°C / 20°C	10	1.46	1.28	2.74	5.23	7.97

other words, the high day temperature of 20°C or 30.3°C could eliminate the sugar that had accumulated as a result of low night temperature.

(2) Effect of high temperature and alternating day/night temperature following vernalization on bolting

By the time of the final observation on April 9, 1983, it was seen that when plants had been treated with cold temperature (5°C) for 20 days, percentage of bolting plants was a little lower during the early stage when cold treatment was followed by a continuous temperature of 30°C or 30°C day/20°C night than when cold was followed with only 20°C (Table 5–4). However, on April 9, end of experiment all treatments were 100% or nearly 100% of plants bolted. It means that although heat treatments caused a delay in flowering implying unfavorable flower development conditions, the prolonged 5°C for 20 days treatment had caused complete vernalization and neither subsequent 30°C nor 30°C day/20°C night treatments could reverse the effect.

Table 5–4. *Effect of high temperature and alternating day/night temperature following cold temperature treatment on bolting*

Treatments	No. of plants treated	% of bolting plants*						
		3/10	3/15	3/20	3/25	3/30	4/4	4/9**
5°C (20 days) → 20°C	30	26.7	50	63.3	83.3	93.3	100	100
5°C (20 days) → 30°C	36	22.2	30.6	52.8	66.7	72.2	88.9	97.2
5°C (20 days) → 20°C/30°C	30	23.3	26.6	60.0	66.7	73.3	90.0	100
5°C (10 days) → 20°C	39	0	0	5.1	12.8	15.4	25.6	43.6
5°C (10 days) → 30°C	52	0	0	0	3.8	19.2	19.2	23.1
5°C (10 days) → 20°C/30°C	28	0	0	0	3.6	17.9	21.4	28.6

*Mean temperature during planted in glasshouse: 20.94°C

**Plants which had flower initiation included (checked by microscope)

Bolting was later and percentage of bolting plants was lower for plants treated with cold temperature for 10 days than for plants treated with cold for 20 days when cold treatment was followed by 20°C. When cold temperature treatment of only

10 days was followed by the other two heat treatments (30°C or 30°C day/20°C night), percentage of bolting plants was lower. Evidently the period of cold treatment was too brief for complete vernalization to take place. Thus reversal of vernalization is possible when high temperatures follow an incomplete vernalization.

Suzuki (1981) indicated that alternating day/night temperature (30°C/20°C or 30°C/15°C) after a complete vernalization in radish could not cause devernalization. Purvis and Gregory (1952) pointed out that in winter rye, with increasing duration of vernalization, the reversal becomes progressively less. The vernalized condition thus becomes increasingly stable. After 12 weeks of vernalization, heat treatment at 35°C had no effect, even when given for 5 days; no devernalization could occur.

Vernalizing and devernalizing temperatures are different. Temperatures for devernalization, usually range from 25 to 40°C but for some plants made neutral temperatures can have this effect. For example, devernalization can occur at 17°C in winter rye (Purvis and Gregory 1952), 17 to 18°C in sugarbeet (Owen etc. 1940), and 20°C in *Hyoscyamus, Arabidopsis* (Melchers and Lang 1947), and radish (Suzuki 1980). It is interesting to note that the potential for devernalization by heat varies inversely with the completeness of the preceding vernalization treatment. If the vernalization process has been completed reversal by heat becomes impossible.

The carbohydrate contents of radish after complete vernalization subsequent high temperature or alternating day/night temperature treatment showed that high levels of total sugar and non-reducing sugar remained, but no significant difference was found among the 20°C, 30°C, or 30°C/20°C treatment. (Table 5–5). Plants treated with 20°C temperature seemed to have higher non-reducing sugar content. High temperature following incomplete vernalization gave low levels of total sugar, non-reducing sugar, and reducing sugar content as well as a higher starch content.

The results show clearly that the thermo induced vernalized state, if completely established, has a high level of non-reducing sugar and total sugar content and maintains stability even when subsequently subjected to high temperature or alternating day/night temperatures. In other words, stability of thermo induction depends upon the continuous maintenance of the level

Table 5–5. *Carbohydrate content (% dry matter) of radish seedling under various temperature treatments*

Treatments	Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrate
5°C (20 days) → 20°C	0.27	4.78	5.05	5.89	10.94
5°C (20 days) → 30°C	1.99	3.46	5.45	5.01	10.46
5°C (20 days) → 20°C/30°C	0.27	5.77	6.04	4.99	11.03
5°C (10 days) → 20°C	0.09	1.91	2.00	5.29	7.29
5°C (10 days) → 30°C	0.98	1.61	1.70	5.22	6.92
5°C (10 days) → 20°C/30°C	0.09	1.69	1.78	5.26	8.04

of total sugar content during the subsequent high temperature treatment. The high sugar content is stabilized after complete vernalization, and will not be reversed even under subsequent high temperatures. These conditions might imply that high sugar content is necessary for promoting flower initiation and development. When the sugar level is too low for flower development, high temperature can convert this low level of sugar into starch, and devernalization thus occurs.

The results of the experiments show the possibility of reversing vernalization by high temperature treatment. The effect of alternating high day/low night temperature during the course of vernalization, and the effect of high temperature or alternating day/night temperature following vernalization must be clearly distinguished.

First, the test alternating high day temperature with low night temperature during vernalization showed that temperature as low as 20°C in the daytime will reverse the effect of the low night temperature. Second, the stability of the vernalized condition appears to vary according to the degree of vernalization. A completely vernalized condition will not be reversed by subsequent high temperature of 30.3°C or alternating day/night temperature of 20°C/30°C, but an incompletely vernalized condition can certainly be annulled by subsequent high temperature or alternating day/night temperature, and an increase in vegetative development occurs as well.

The following carbohydrate metabolic changes associated

with devernalization treatment were observed: First, the level of sugar contents increased with the duration of vernalization. However, when low night temperatures were alternated with higher day temperatures during the course of vernalization, even the optimal growth temperature of 20°C could nullify the sugar-raising of effect lower night temperature. Accordingly, the accumulation of sugar contents could not reach the level required for flower initiation and development. In the subtropics, the relatively high day temperature during the winter season is one of the major factors preventing bolting in plants that require cold.

Second, it was clear that the stability of vernalization depends upon the degree of vernalization. A complete vernalization requires a stable high level of sugar contents which cannot be annulled by subsequent high temperature or alternating day/night temperatures. Thus the high level of sugar contents might be responsible for the initiation and development of flowering. Incomplete, unstable vernalization in the tropics is mainly due to insufficient accumulation of sugars in plant tissues. The subtropical environment does not provide a continuously low enough temperature for complete vernalization to take place, especially as winter season temperatures fluctuate.

D. Summary

Ta-Mei-Fua radish must be exposed to a temperature of 5°C for a period of 20 days to achieve complete vernalization. Exposure to 5°C for only 10 days resulted in incomplete vernalization. When temperatures varied from day to night during vernalization, the higher daytime temperature erased the vernalizing effect of low night temperature. It was shown that the higher daytime temperature of 20 or 30.3°C eliminated the sugar formed during the cooler nights.

After an uninterrupted period of vernalization, neither subsequent high temperature nor alternating high day/low night temperature could reverse the complete vernalization. It was also shown that a completely vernalized plant maintains a stable high sugar content which neither high temperature nor day/night temperature variation could reduce. Thus, a high sugar level in vernalized plants might account for the hastening of flower initiation and development.

Ⅳ. Conclusions

Most cruciferous crops grown in the tropics cannot flower or if they do, flower incompletely because of insufficiently low temperatures. Thus, seed production of crucifers in the tropics is difficult. Although most types can flower in the cool tropical highland, their reproductive cycles are slowed down so that the seeding stage overlaps with the rainy season during May and June in Taiwan. In terms of the phasic development theory (Lysenko 1932; Shinohara 1959), the low temperature requirements of the first phase (thermo-phase) of development can be satisfied by planting under high altitude conditions, but those same conditions present the problems of rainfall during seed production in the second phase (photo-phase) of development.

In the tropics and subtropics, only varieties exhibiting a shallow first-and second-phase could exist under natural conditions. Varieties which require extremely deep second-phase, or basic-vegetative and first-phase, could not be multiplied in the tropics or subtropics. For cabbage and kohlrabi, only tropical type varieties with minimal cold requirements such as var. Yehsen and var. Kimmen-Early, respectively, can flower in Taiwan. As climate generally varies from year to year, a variety may not normally attain 100% flowering during seed production in the tropics. In warm years, a high proportion of non-bolting plants will occur. Owing to the responses of environment, a variety with a high percentage of prematurely bolting plant will gradually degenerate. The author (1982) conducted flowering studies on 30 varieties of radish at Fengshan Tropical Horticultural Experiment Station and found that most local varieties flowered the same year of sowing or in January and February of the following year except Ta-Mei-Fua Late. The results indicated that after a long period of seed production, low temperature requirement of the first- and second-phase became very shallow or lacking for most cultivars. Premature bolting then significantly reduced radish production. Methods of promoting 100% flowering for seed production, and promoting stock-seed plants to flower for breeding purposes are two important tasks for cruciferae improvement work and seed production in the subtropics and tropics.

In this study, it was found that seeds of cabbage and kohlrabi could be harvested before the rainy season if the plants were vernalized in the cool highlands and then transplanted to the warmer and drier lowlands for seed production. A complete vernalization treatment shortened the time to flowering and also enhanced flower organ development and seed quality of most cruciferous crops studied.

Although high temperatures normally induce devernalization, the present experiement showed that, after being exposed to low temperatures for flower formation, plants could proceed through flowering and seed ripening under mild weather conditions. The results of the present experiment showed that such a method was possible for cabbage and kohlrabi seed production, and that early or mid-December was the optimum transplanting time for plants which had been completely vernalized. This method could also be used practically for multiplying the parent stock-seed of F_1 hybrids.

Stock-seed plants of kohlrabi and radish could be treat low temperature with high humidity for flower induction. Peking Late kohlrabi exposed to 8°C under total darkness for six weeks, and Ta-Mei-Fua radish exposed to 9°C and 12 hr day length for three weeks were able to complete phasic development and produce seeds for stock seed use. Inducing stock-seed plants to flower is an essential step in breeding slow bolting varieties in the tropics or subtropics. It is thus possible not only to select and develop slow bolting varieties to prevent premature bolting, but also to employ a way for multiplying genetic materials from a temperate country in the tropics.

Results from the present studies on the morphological changes in the shoot apex and changes in sugar content during vernalization showed that both green-plant vernalization and seed-vernalization types had the same metabolic pattern: increase in sugar content and high total sugar/N ratio just before flower bud differentiation, and decrease in sugar content after flower bud formation and bolting. The differences in the low temperature response of radish and kohlrabi seeds are thus determined by the sugar content of the seeds at the time of exposure to low temperature. Total sugar and reducing sugar contents increased and non-reducing sugar decreased when radish seeds were exposed to low temperatures, while no such changes were noted for kohlrabi seeds which were given the same treatment.

Alternating day/night temperatures during vernalization, and high temperatures or alternating day/night temperatures following vernalization, had varying effects on the reversibility of vernalization. In the present study, day temperature as low as 20°C during the course of vernalization was found to reverse the low night temperature effect, but after complete vernalization, subsequent high temperatures or alternating day/night temperatures did not alter the vernalization process. Subsequent high temperatures after incomplete vernalization reversed the effect of cold temperature.

The metabolic changes of carbohydrates with devernalization were

found to be related to the level of total sugar accumulation. Under alternating high day/low night temperatures during vernalization and when incomplete vernalization was followed by high temperatures, the sugar content was insufficient for floral development. On the contrary, after complete vernalization the quantity of sugar content was irreversibly higher and stable, and subsequent high temperatures or alternating high day/low night temperatures did not induce devernalization.

From a genetical point of view, stock-seed plant vernalization is an essential step to prevent degeneration of varieties in the tropics. Using maternal line selection, the process of stock-seed plant vernalization for seed production is proposed as follows (Fig. 6–1):

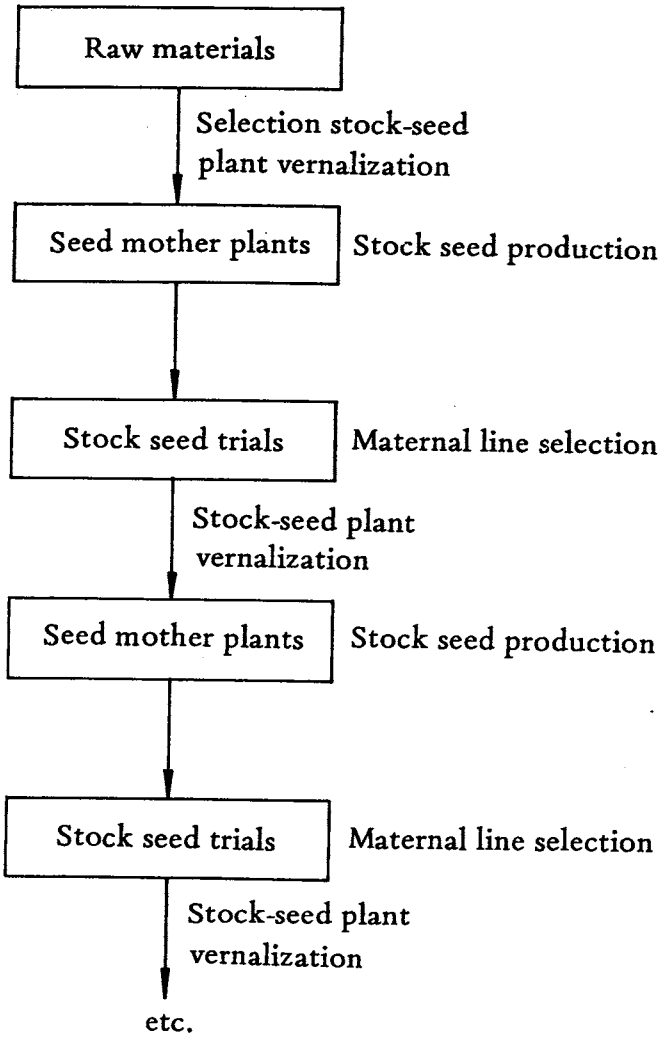

Fig. 6–1. Procedure of stock-seed plant vernalization for seed production under maternal line selection program.

The so-called "partial vernalization method" developed by Shinohara (1959) was practically used in selecting non-bolting types or slow bolting types in the temperate zone. This method is also applicable to select slow bolting seed vernalization type in the tropics. However, when applied to radish the root system is often injured after seed vernalization, causing abnormal roots or branching roots, and it takes one more year for root-type selection. In the case of stock-seed-plant revernalization, selection for horticultural traits and seed production can be finished in the same year. This method can be applied to both seed- and green-plant vernalization types.

The use of stock-seed plant vernalization, with plants vernalized in the highland and then transplanted to the lowland for seed production, instead of the traditional method is effective in improving the stability of seed production and in the preventing the degeneration of varieties in the tropics. A summary of seed production pattern of cruciferous crops in Taiwan is given in Table 6–2.

In the traditional radish seed production, seeds are usually sown in the lowland from the end of November to the beginning December. Roots of most cultivars are difficult to select at maturity because plants bolt during the winter period. For selecting stock-seed plants, whether summer-sown radish (include 40–50 day and 50–60 day groups) or autumn-sown radish (include 60–70 day, non-bolting groups), the materials should be grown in the field during the regular production season. At time of root maturity, selection for desirable root characteristics should be carried out. In addition the selected stock plant should be vernalized for a certain period before they are transplanted to the field for stock seed production. The maintenance of selected radish plants through the Taiwan summer is difficult because of severe root rot in hot weather. By adopting the proposed method in Table 6–1, radish could be sown in the summer for desirable root characters selection and used as stock-seed plants. Those plants allowed to pass through the hot summer period by vernalization. Winter radish which does not bolt in the winter could be sown either in fall or winter and stok-seed plants selected at maturity. The "root-to-seed" method achieved through selection and stock-seed plants vernalization are two important steps for stock seed production in the tropics.

In contract to stock seed production, commercial seed production of radish can be done without regard for root characters. The emphasis should be given to economical and stable seed production with high yield. As in the traditional method, stock seeds may be direct-seeded

Table 6–2. *Traditional and proposed seed production patterns of cruciferous crops in Taiwan*

Crops	Groups	Main varieties	Traditional	Proposal
Radish	A. Early bolting	Thai radish		1) Stock-seed plant vernalization for stock seed production (sown in summer or fall–lowland)
	B. 40–50 day group	40-day Kan-naki 40-day radish Ta-zer-pa-chu Ma-er-chou	Sown in lowland (winter)	
	C. 50–60 day group	60-day Kan naki Ta paichi Tang-kua-pei Tou-chou-Early Nan-pan-chou 50–60 day kan-rah 50-day Fu-non		2) Using stock seed for commercial seed production in lowland or 500–800 m highland
	D. 60–70 day group	Meifua-kanrah Meifua-kirah Ta-Mei-Fua Minowase		
	E. Non-bolting group	Ta-Mei-Fua Late Shogoin Futori Ookura	Sown in 500–800 m highland (winter)	
Cabbage	Tropical group	Yehsen Karah Ta-parhn T.S.S. No. 1	Sown in lowland (fall)	1) Stock-seed plant vernalization for stock seed production (sown in summer or fall–lowland) 2) Plant vernalize in highland and transplant to lowland
	Non-bolting group	Chu-chiou Chu-hsiah Hsiah-fuon 60-day cabbage Chiou-wang Chaou-chiou	– – – – – –	
Kohlrabi	Tropical group	Kimmen Early	–	The same as cabbage
	Non-bolting group	Peking Late Winner	– –	

in the lowland or at 500–800m highland from the end of November to the beginning of December. The technique of "seed-vernalization" can be practically applied to this method to promote flowering. This production uses direct seeding and is called "seed to seed" method.

Cabbage and kohlrabi are either of the tropical group or the non-bolting group. Seeds could be sown in the summer or fall season and stock-seed plants may be selected at the time of heading (cabbage) or swollen stem (kohlrabi) maturity. The selected stock-seed plants can then be kept in a high humidity room at 8°C for a period of vernalization and then seeds may be produced by "plant-to-seed" method. This system enhances the success of maintaining the stock-seed plants selected in the hot summer season. For commercial seed production, the stock seed plants can be grown at high altitude in the summer or fall season. Through a period of vernalization, plants will finish the thermo-phase and seed production can then be subsequently done in the warm and dry lowland. This seed production method stabilizes the seed yield and also increases seed production potential of slow-bolting varieties in the tropics.

High day temperatures may naturally nullify the effect of vernalization. In seed production, care should to taken to find areas with lower diurnal temperature variation to prevent devernalization. Where vernalization is complete, high temperatures fail to reverse the action. It is implied that the requirement of stock-seed plant vernalization, or vernalization in highland areas, should be completely finished so that the stock seed production and commercial seed production in the tropics will be smooth.

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